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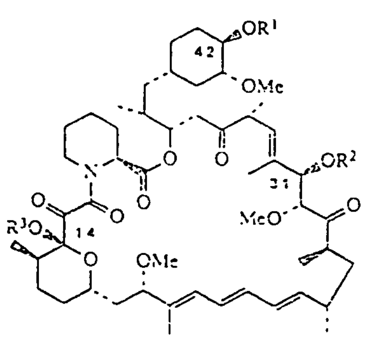
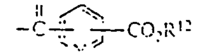
(54) Title: CARBOXYLIC ACID ESTERS OF RAPAMYCIN



(I)

R^4 is $-[\overset{O}{\overset{\|}{C}}(CH_2)_mCH(R^5)(CH_2)_nN(R^6)]_pCO_2R^7$ (a)

$-\overset{O}{\overset{\|}{C}}-(CH_2)_tX(CH_2)_uCO_2R^{11}$ (b)



(c)

X is $-\underset{R^{14}}{\overset{R^{13}}{C}}-$, O, (d)

$[\overset{O}{\overset{\|}{C}}(CH_2)_mCH(R^5)(CH_2)_nN(R^6)]$ (e)

(57) Abstract

A compound of structure (I), wherein R^1, R^2, and R^3 are each, independently, hydrogen, or R^4; R^4 is (a), (b), or (c); R^5 is hydrogen, alkyl, aralkyl, $-(CH_2)_qCO_2R^8$, $-(CH_2)_rNR^9CO_2R^{10}$, carbamylalkyl, aminoalkyl, hydroxyalkyl, guanylalkyl, mercaptoalkyl, alkylthioalkyl, indolylmethyl, hydroxypehnylmethyl, imidazoylmethyl or phenyl which is optionally mono-, di-, or tri-substituted with a substituent selected from alkyl, alkoxy, hydroxy, cyano, halo, nitro, carbalkoxy, trifluoromethyl, amino, or a carboxylic acid; R^6 and R^9 are each, independently, hydrogen, alkyl, or aralkyl; R^7, R^8, and R^{10} are each, independently, alkyl, aralkyl, fluorenylmethyl, or phenyl which is optionally mono-, di-, or tri-substituted; R^{11} and R^{12} are each, independently, alkyl, aralkyl, or phenyl which is optionally mono-, di-, or tri-substituted; X is (d), O, or S; R^{13} and R^{14} are each, independently, hydrogen or alkyl; Y is CH or N; m is 0-4; n is 0-4; p is 1-2; q is 0-4; r is 0-4; t is 0-4; u is 0-4; wherein R^5, R^6, m, and n are independent in each of (e) subunits when p=2; or a pharmaceutically acceptable salt thereof, with the proviso that R^1, R^2, and R^3 are not all hydrogen, further provided that R^1, R^2 and R^3 are not all (a), and still further provided that t and u are not both 0 when X is O or S, which by virtue of its immuno-suppressive activity is useful in treating transplantation rejection, host vs. graft disease, autoimmune diseases, and diseases of inflammation, and by virtue of its antifungal activity is useful in treating fungal infections.

* See back of page

\+ DESIGNATIONS OF "SU"

Any designation of "SU" has effect in the Russian Federation. It is not yet known whether any such designation has effect in other States of the former Soviet Union.

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CARBOXYLIC ACID ESTERS OF RAPAMYCIN

BACKGROUND OF THE INVENTION

This invention relates to novel esters of rapamycin and a method for using them in the treatment of transplantation rejection, host vs. graft disease, autoimmune diseases, diseases of inflammation, and fungal infections.

Rapamycin is a macrocyclic triene antibiotic produced by Streptomyces hygroscopicus, which was found to have antifungal activity, particularly against Candida albicans, both in vitro and in vivo [C. Vezina et al., J. Antibiot. 28, 721 (1975); S.N. Seghal et al., J. Antibiot. 28, 727 (1975); H. A. Baker et al., J. Antibiot. 31, 539 (1978); U.S. Patent 3,929,992; and U.S. Patent 3,993,749].

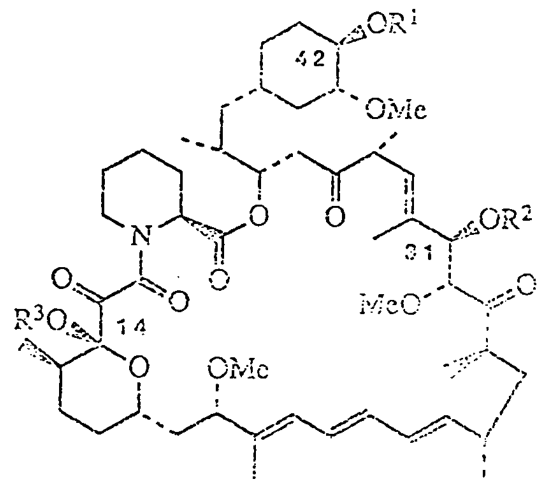
Rapamycin alone (U.S. Patent 4,885,171) or in combination with picibanil (U.S. Patent 4,401,653) has been shown to have antitumor activity. R. Martel et al. [Can. J. Physiol. Pharmacol. 55, 48 (1977)] disclosed that rapamycin is effective in the experimental allergic encephalomyelitis model, a model for multiple sclerosis; in the adjuvant arthritis model, a model for rheumatoid arthritis; and effectively inhibited the formation of IgE-like antibodies.

The immunosuppressive effects of rapamycin have been disclosed in FASEB 3, 3411 (1989), rapamycin has been shown to be effective in inhibiting transplant rejection (U.S. Patent Application Ser. No. 362,544 filed June 6, 1989). Cyclosporin A and FK-506, other macrocyclic molecules, also have been shown to be effective as immunosuppressive agents, therefore useful in preventing transplant rejection [FASEB 3, 3411 (1989); FASEB 3, 5256 (1989); and R. Y. Calne et al., Lancet 1183 (1978)].

Mono- and diacylated derivatives of rapamycin (esterified at the 28 and 43 positions) have been shown to be useful as antifungal agents (U.S. Patent 4,316,885) and used to make water soluble prodrugs of rapamycin (U.S. Patent 4,650,803). Recently, the numbering convention for rapamycin has been changed; therefore according to Chemical Abstracts nomenclature, the esters described above would be at the 31- and 42- positions.

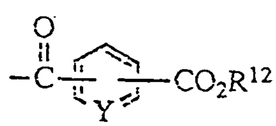
DESCRIPTION OF THE INVENTION

This invention provides derivatives of rapamycin which are useful as immunosuppressive, anti-inflammatory, and antifungal agents having the structure



wherein R^1, R^2, and R^3 are each, independently, hydrogen, or R^4;

R^4 is $-[\overset{O}{\overset{\|}{C}}(CH_2)_m\underset{R^5}{\underset{|}{CH}}(CH_2)_n\underset{R^6}{\underset{|}{N}}]_pCO_2R^7$, $-\overset{O}{\overset{\|}{C}}-(CH_2)_tX(CH_2)_uCO_2R^{11}$, or



R^5 is hydrogen, alkyl of 1-6 carbon atoms, aralkyl of 7-10 carbon atoms, $-(CH_2)_qCO_2R^8$, $-(CH_2)_rNR^9CO_2R^{10}$, carbamylalkyl of 2-3 carbon atoms, aminoalkyl of 1-4 carbon atoms, hydroxyalkyl of 1-4 carbon atoms, guanylalkyl of 2-4 carbon atoms, mercaptoalkyl of 1-4 carbon atoms, alkylthioalkyl of 2-6 carbon atoms, indolylmethyl, hydroxyphenylmethyl, imidazolylmethyl or phenyl which is optionally mono-, di-, or tri-substituted with a substituent selected from alkyl of 1-6 carbon atoms, alkoxy of 1-6 carbon atoms, hydroxy, cyano, halo, nitro, carbalkoxy of 2-7 carbon atoms, trifluoromethyl, amino, or a carboxylic acid;

R^6 and R^9 are each, independently, hydrogen, alkyl of 1-6 carbon atoms, or aralkyl of 7-10 carbon atoms;

R^7, R^8, and R^{10} are each, independently, alkyl of 1-6 carbon atoms, aralkyl of 7-10 carbon atoms, fluorenylmethyl, or phenyl which is optionally mono-, di-, or tri-substituted with a substituent selected from alkyl of 1-6 carbon atoms, alkoxy of 1-6 carbon atoms, hydroxy, cyano, halo, nitro, carbalkoxy of 2-7 carbon atoms, trifluoromethyl, amino, or a carboxylic acid;

R^{11} and R^{12} are each, independently, alkyl of 1-6 carbon atoms, aralkyl of 7-10 carbon atoms, or phenyl which is optionally mono-, di-, or tri-substituted with a substituent selected from alkyl of 1-6 carbon atoms, alkoxy of 1-6 carbon atoms, hydroxy, cyano, halo, nitro, carbalkoxy of 2-7 carbon atoms, trifluoromethyl, amino, or a carboxylic acid;

X is $-\underset{R^{14}}{\overset{R^{13}}{\underset{|}{\overset{|}{C}}}}-$, O, or S;

R^{13} and R^{14} are each, independently, hydrogen or alkyl of 1-6 carbon atoms;

Y is CH or N;

m is 0 - 4;

n is 0 - 4;

p is 1 - 2;

q is 0 - 4;

r is 0 - 4;

t is 0 - 4;

u is 0 - 4;

wherein R^5, R^6, m, and n are independent in each of the $[\overset{O}{\overset{\|}{C}}(CH_2)_m\underset{R^5}{\underset{|}{CH}}(CH_2)_n\underset{R^6}{\underset{|}{N}}]$ subunits when p = 2;

or a pharmaceutically acceptable salt thereof, with the proviso that R^1, R^2, and R^3 are not all hydrogen, further provided that R^1, R^2, and R^3 are not all

$$-[\overset{O}{\overset{\|}{C}}(CH_2)_m\underset{R^5}{\underset{|}{CH}}(CH_2)_n\underset{R^6}{\underset{|}{N}}]_pCO_2R^7$$

, and still further provided that t and u are not both 0 when X is O or S.

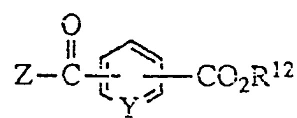
Of the compounds when R^4 is $-[\overset{O}{\overset{\|}{C}}(CH_2)_m\underset{R^5}{\underset{|}{C}}H(CH_2)_n\underset{R^6}{\underset{|}{N}}]_pCO_2R^7$,

preferred members are those in which m = 0, n = 0, and p = 1; m = 0, n = 0, and p = 2; n = 0, and R^5 is $-(CH_2)_qCO_2R^8$; m = 0, n = 0, and R^5 is $-(CH_2)_rNR^9CO_2R^{10}$; and m = 0, n = 0, and R^5 is hydrogen. Preferred compounds also include those members in which R^4 is $-\overset{O}{\overset{\|}{C}}-(CH_2)_tX(CH_2)_uCO_2R^{11}$.

The pharmaceutically acceptable salts may be formed from inorganic cations such as sodium, potassium, and the like; mono-, di-, and trialkyl amines of 1-6 carbon atoms, per alkyl group and mono-, di-, and trihydroxyalkyl amines of 1-6 carbon atoms per alkyl group; and organic acids such as acetic, lactic, citric, tartaric, succinic, maleic, malonic, gluconic, and the like. Preferred basic salts are formed from sodium cations and tris(hydroxymethyl)methylamine.

The compounds of this invention can be prepared by acylating rapamycin with an acylating agent having the general structures

$Z-[\overset{O}{\overset{\|}{C}}(CH_2)_m\underset{R^5}{\underset{|}{C}}H(CH_2)_n\underset{R^6}{\underset{|}{N}}]_pCO_2R^7$, $Z-\overset{O}{\overset{\|}{C}}-(CH_2)_tX(CH_2)_uCO_2R^{11}$, or



where Z is OH in the presence of a coupling reagent, such as dicyclohexylcarbodiimide. The compounds of this invention also can be prepared using an anhydride or a mixed anhydride of the above described carboxylic acid as the acylating species. Alternatively, the acylating species can be an acid halide, where Z can be Cl, Br, or I. The acylating groups used to prepare the compounds of this invention are commercially available or can be prepared by methods that are disclosed in the literature.

Where it is desired to prepare acyl derivatives having two or three different R^4 groups then sequential acylation may be performed using appropriate acylating agents as defined above, if necessary isolating the desired product by appropriate purification

techniques. In general the 42-position is acylated first and such a monoacylated product may be isolated prior to the second acylation and so forth. Appropriate protecting groups may be used to block any position where acylation is not required.

Immunosuppressive activity was evaluated in an in vitro standard pharmacological test procedure to measure lymphocyte proliferation (LAF) and in two in vivo standard pharmacological test procedures. The first in vivo procedure was a popliteal lymph node (PLN) test procedure which measured the effect of compounds of this invention on a mixed lymphocyte reaction and the second in vivo procedure evaluated the survival time of a pinch skin graft.

The comitogen-induced thymocyte proliferation procedure (LAF) was used as an in vitro measure of the immunosuppressive effects of representative compounds. Briefly, cells from the thymus of normal BALB/c mice are cultured for 72 hours with PHA and IL-1 and pulsed with tritiated thymidine during the last six hours. Cells are cultured with and without various concentrations of rapamycin, cyclosporin A, or test compound. Cells are harvested and incorporated; radioactivity is determined. Inhibition of lymphoproliferation is assessed in percent change in counts per minute from non-drug treated controls. The results are expressed by the following ratio, or as the percent inhibition of lymphoproliferation of 1 μM.

$$\frac{{}^{3}\text{H-control thymus cells} - \text{H}^{3}\text{-rapamycin-treated thymus cells}}{{}^{3}\text{H-control thymus cells} - \text{H}^{3}\text{-test compound-treated cells}}$$

A mixed lymphocyte reaction (MLR) occurs when lymphoid cells from genetically distinct animals are combined in tissue culture. Each stimulates the other to undergo blast transformation which results in increased DNA synthesis that can be quantified by the incorporation of tritiated thymidine. Since stimulating a MLR is a function of disparity at Major Histocompatibility antigens, an in vivo popliteal lymph node (PLN) test procedure closely correlates to host vs. graft disease. Briefly, irradiated spleen cells from BALB/c donors are injected into the right hind foot pad of recipient C3H mice. The drug is given daily, p.o. from Day 0 to Day 4. On Day 3 and Day 4, tritiated thymidine is given i.p., b.i.d. On Day 5, the hind popliteal lymph nodes are removed and dissolved, and radioactivity counted. The corresponding left PLN serves as the control for the PLN from the injected hind foot. Percent suppression is calculated using the non-drug treated animals as allogenic control. Rapamycin at a dose of 6 mg/kg, p.o. gave 86% suppression, whereas cyclosporin A at the same dose gave 43% suppression. Results are expressed by the following ratio:

$$\frac{^{3}\text{H-PLN cells control C3H mouse} - {}^{3}\text{H-PLN cells rapamycin-treated C3H mouse}}{^{3}\text{H-PLN cells control C3H mouse} - {}^{3}\text{H-PLN cells test compound-treated C3H mouse}}$$

The second in vivo test procedure is designed to determine the survival time of pinch skin graft from male DBA/2 donors transplanted to male BALB/c recipients. The method is adapted from Billingham R.E. and Medawar P.B., J. Exp. Biol. 28:385-402, (1951). Briefly, a pinch skin graft from the donor is grafted on the dorsum of the recipient as a homograft, and an autograft is used as control in the same region. The recipients are treated with either varying concentrations of cyclosporin A as test control or the test compound, intraperitoneally. Untreated recipients serve as rejection control. The graft is monitored daily and observations are recorded until the graft becomes dry and forms a blackened scab. This is considered as the rejection day. The mean graft survival time (number of days ± S.D.) of the drug treatment group is compared with the control group.

The following table summarizes the results of representative compounds of this invention in these three standard test procedures.

TABLE 1

Compound	LAF* (ratio)	PLN* (ratio)	Skin Graft (days ± SD)
Example 1	1.8	0.61	12.0 ± 1.6
Example 2	0.33	0.62	11.5 ± 0.6
Example 3	0.20	+	9.0 ± 0.9
Example 4	4.9	0.18	12.3 ± 0.5
Example 5	0.006	+	8.8 ± 0.9
Example 6	5.4	0.33	11.5 ± 3.5
Example 7	3% at 1μM**	+	7.7 ± 1.5
Example 8	0.03	0.41	+
Example 9	0.96	1.34	10.3 ± 0.8
Example 10	2.0	0.96++	12.7 ± 1.2
Example 11	0.004	+	10.5 ± 1.3
Example 12	19.8	-2.87	12.0 ± 2.0
Example 13	22% at 1μM**	+	7.0 ± 0.6
Example 14	0.37	+	8.2 ± 1.2
Example 15	0.9	0.69	10.7 ± 1.2

TABLE 1 (Continued)

Compound	LAF* (ratio)	PLN* (ratio)	Skin Graft (days ± SD)
Example 16	3.27	1.04##	12.7 ± 0.9
Example 17	0.56	1.68###	10.2 ± 1.7
Example 18	0.02	1.11##	8.0 ± 1.7
Example 19	0.01	0.48	8.0 ± 0.9
Example 20	0.97	0.70	9.3 ± 1.6
Example 21	0.22	-1.93	12.0 ± 1.7
Example 22	0.22	0.41	10.2 ± 1.2
Example 23	0.18	0.39	10.8 ± 0.8
Example 24	0.00	0.09	7.8 ± 1.7
Rapamycin	1.0	1.0	12.0 ± 1.7

* Calculation of ratios was described supra.

** Result expressed as percent inhibition of lymphoproliferation at 1 μM.

\+ Not evaluated

++ Results obtained using cremophore/ethanol as a vechicle for administration. Ratios of 0.33 and 1.07 were also obtained using carboxymethyl cellulose as a vehicle for administration.

Results obtained using cremophore/ethanol as a vechicle for administration. Ratios of 0.20 and 1.08 also were obtained using carboxymethyl cellulose as a vehicle for administration.

A ratio of 0.42 also was obtained for this compound.

The results of these standard pharmacological test procedures demonstrate immunosuppressive activity both in vitro and in vivo for the compounds of this invention. Positive ratios in the LAF and PLN test procedures indicate suppression of T cell proliferation. As a transplanted pinch skin grafts are typically rejected within 6-7 days without the use of an immunosuppressive agent, the increased survival time of the skin graft when treated with the compounds of this invention further demonstrates their utility as immunosuppressive agents. While it appears that the compound disclosed by Examples 12 and 21 may cause T cell proliferation in the PLN test procedure, it is believed a negative ratio in this test procedure coupled with an increased survival time observed in the skin graft test procedure indicates a proliferation of T$_{suppressor}$ cells,

which are implicated in suppressing the immune response. (see, I. Roitt et al. Immunology, C.V.Moseby Co. 1989, p 12.8-12.11).

Antifungal activity of the compounds of this invention was measured against 5 strains of Candida albicans using a plate test procedure for measurement of inhibition. The following represents the typical procedure used. Compound to be tested was placed on sterile dried 1/4" plate disks, and allowed to dry. Agar plates were seeded with fungi and allowed to solidify. The impregnated disks were placed on the seeded Agar surface and incubated for the time required for the particular culture. Results are expressed in MIC (μg/ml) to inhibit growth. The results of this test procedure showed that the compounds of this invention have antifungal activity; however, it was surprising that the compounds of this invention were less active than the parent compound, rapamycin.

Table 2*

Strain of Candida albicans

Compound	ATCC 10231	ATCC 38246	ATCC 38247	ATCC 38248	3669
Example 1	> 0.4	> 0.4	> 0.4	>0.4	>0.4
Example 2	0.1	0.2	0.2	0.2	0.1
Example 3	0.4	> 0.4	> 0.4	>0.4	0.4
Example 4	0.1	0.4	0.1	0.1	0.2
Example 5	> 0.4	> 0.4	> 0.4	>0.4	>0.4
Example 6	0.1	> 0.4	0.2	0.4	>0.4
Example 7	+	+	+	+	+
Example 8	> 0.4	> 0.4	> 0.4	>0.4	>0.4
Example 9	0.4	> 0.4	0.4	>0.4	>0.4
Example 10	0.2	> 0.4	0.2	0.4	0.4
Example 11	> 0.4	> 0.4	> 0.4	>0.4	>0.4
Example 12	0.2	> 0.4	0.1	0.2	0.4
Example 13	> 0.4	> 0.4	> 0.4	>0.4	>0.4
Example 14	> 0.4	> 0.4	> 0.4	>0.4	>0.4
Example 15	> 0.4	0.4	> 0.4	0.4	0.4
Example 16	0.2	0.1	0.4	0.1	0.1
Example 17	> 0.4	0.2	> 0.4	0.2	0.4
Example 18	0.4	> 0.4	> 0.4	>0.4	>0.4
Example 19	0.4	> 0.4	0.4	>0.4	>0.4

Table 2* (Continued)

Strain of Candida albicans

Compound	ATCC 10231	ATCC 38246	ATCC 38247	ATCC 38248	3669
Example 20	0.1	0.4	0.1	0.1	0.2
Example 21	0.4	> 0.4	0.4	>0.4	>0.4
Example 22	0.2	> 0.4	0.2	0.4	>0.4
Example 23	0.1	> 0.4	0.2	0.4	>0.4
Example 24	> 0.4	> 0.4	>0.4	>0.4	>0.4
Rapamycin	0.003	0.025	0.003	0.006	0.025

* expressed as MIC (μg/ml)

+ not evaluated

Based on the results of these standard pharmacological test procedures, the compounds are useful in the treatment of transplantation rejection such as, heart, kidney, liver, bone marrow, and skin transplants; autoimmune diseases such as, lupus, rheumatoid arthritis, diabetes mellitus, myasthenia gravis, and multiple sclerosis; and diseases of inflammation such as, psoriasis, dermatitis, eczema, seborrhea, inflammatory bowel disease; and fungal infections.

The compounds may be administered neat or with a pharmaceutical carrier to a mammal in need thereof. The pharmaceutical carrier may be solid or liquid.

A solid carrier can include one or more substances which may also act as flavoring agents, lubricants, solubilizers, suspending agents, fillers, glidants, compression aids, binders or tablet-disintegrating agents; it can also be an encapsulating material. In powders, the carrier is a finely divided solid which is in admixture with the finely divided active ingredient. In tablets, the active ingredient is mixed with a carrier having the necessary compression properties in suitable proportions and compacted in the shape and size desired. The powders and tablets preferably contain up to 99% of the active ingredient. Suitable solid carriers include, for example, calcium phosphate, magnesium stearate, talc, sugars, lactose, dextrin, starch, gelatin, cellulose, methyl cellulose, sodium carboxymethyl cellulose, polyvinylpyrrolidine, low melting waxes and ion exchange resins.

Liquid carriers are used in preparing solutions, suspensions, emulsions, syrups, elixirs and pressurized compositions. The active ingredient can be dissolved or suspended in a pharmaceutically acceptable liquid carrier such as water, an organic solvent, a mixture of both or pharmaceutically acceptable oils or fats. The liquid carrier

can contain other suitable pharmaceutical additives such as solubilizers, emulsifiers, buffers, preservatives, sweeteners, flavoring agents, suspending agents, thickening agents, colors, viscosity regulators, stabilizers or osmo-regulators. Suitable examples of liquid carriers for oral and parenteral administration include water (partially containing additives as above, e.g. cellulose derivatives, preferably sodium carboxymethyl cellulose solution), alcohols (including monohydric alcohols and polyhydric alcohols, e.g. glycols) and their derivatives, and oils (e.g. fractionated coconut oil and arachis oil). For parenteral administration, the carrier can also be an oily ester such as ethyl oleate and isopropyl myristate. Sterile liquid carriers are useful in sterile liquid form compositions for parenteral administration. The liquid carrier for pressurized compositions can be halogenated hydrocarbon or other pharmaceutically acceptable propellent.

Liquid pharmaceutical compositions which are sterile solutions or suspensions can be utilized by, for example, intramuscular, intraperitoneal or subcutaneous injection. Sterile solutions can also be administered intravenously. The compound can also be administered orally either in liquid or solid composition form.

Preferably, the pharmaceutical composition is in unit dosage form, e.g. as tablets or capsules. In such form, the composition is sub-divided in unit dose containing appropriate quantities of the active ingredient; the unit dosage forms can be packaged compositions, for example, packeted powders, vials, ampoules, prefilled syringes or sachets containing liquids. The unit dosage form can be, for example, a capsule or tablet itself, or it can be the appropriate number of any such compositions in package form. The dosage to be used in the treatment must be subjectively determined by the attending physician.

In addition, the compounds of this invention may be employed as a solution, cream, or lotion by formulation with pharmaceutically acceptable vehicles containing 0.1- 5 percent, preferably 2%, of active compound which may be administered to a fungally affected area.

The following examples illustrate the preparation of representative compounds of this invention.

Example 1

Rapamycin-42-ester with N-[(1,1-dimethylethoxy)carbonyl]-glycylglycine

Under anhydrous conditions, a solution of rapamycin (3 g, 3.28 mmole) and N-[(1,1-dimethylethoxy)carbonyl]-glycylglycine (3.04 g, 13.1 mmole) in 40 mL of anhydrous dichloromethane was treated with dicyclohexylcarbodiimide (1.35 g, 6.56 mmole) followed by 4-dimethylaminopyridine (0.8 g, 6.56 mmole). After stirring at ambient temperature for 48 hours, the precipitated solid was collected and washed with dichloromethane. The combined filtrates were absorbed directly onto silica gel Merck 60 by adding the gel and evaporation to dryness. Flash chromatography of the preabsorbed material (using a gradient elution with ethylacetate-toluene from 2:1 to 1:0 v/v) afforded 1.05 g (28.3 %) of the title compound isolated as a three quarter toluene solvate, along with the 31,42-diester of Example 2. HPLC analysis showed that the monoester is a 8.3:1 mixture of two conformers.

^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.46 (m, 9H, $COOBu^t$), 1.654 (s, 3H, $CH_3C{=}C$), 1.751 (s, 3H, $CH_3C{=}C$), 3.14 (s, 3H, CH_3O), 3.33 (s, 3H, CH_3O), 3.36 (s, 3H, CH_3O), 4.18 (d, 1H, C*H*OH), 4.75 (m, 1H, *42*-CHO), 4.79 (s, 1H, OH); High Res. MS (neg. ion FAB) Calcd for $C_{60}H_{93}N_3O_{17}$: 1127.6504, measured mass 1127.6474.

Anal. Calcd for $C_{60}H_{93}N_3O_{17} \cdot 0.75\ PhCH_3$: C, 65.45; H, 8.33; N, 3.51
Found: C, 65,23; H, 8.32; N, 3.86

The following representative compounds can be prepared from rapamycin and the appropriate terminally-N-substituted amino acid by employing the method used to prepare the title compound in Example 1.

Rapamycin-42-ester with N-[(fluorenylmethoxy)carbonyl]-alanylserine
Rapamycin-42-ester with N-[(fluorenylmethoxy)carbonyl]-glycylglycine
Rapamycin-42-ester with N-[(ethoxy)carbonyl]-arginylmethionine
Rapamycin-42-ester with N-[(4'-chlorophenoxy)carbonyl]-histidylarginine
Rapamycin-42-ester with N-[(phenoxy)carbonyl]-tryptophanylleucine
Rapamycin-42-ester with N-[(phenylmethoxy)carbonyl)]-N-methylglycyl-N-ethylalanine

Rapamycin-42-ester with N-[(phenylmethoxy)carbonyl]-N-methyl-ß-alanylphenylalanine
Rapamycin-42-ester with N-[(1,1-dimethylethoxy)carbonyl]-cysteinylglycine

Example 2

Rapamycin-31,42-diester with N-[(1,1-dimethylethoxy)carbonyl]-glycylglycine

The title compound (1.85 g, 42%) was separated from the 42-monoester as described in Example 1 and isolated as a three quarter toluene solvate. HPLC analysis showed that the diester is a 8.1:1 mixture of conformers.
^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.452 (m, 18H, $COOBu^t$), 1.6612 (s, 3H, $CH_3C=C$), 1.7815 (s, 3H, $CH_3C=C$), 3.14 (s, 3H, OCH_3), 3.34 (s, 3H, OCH_3), 3.35 (s, 3H, OCH_3), 4.52 (s, 1H, OH), 4.79 (m, 1H, 42-CHO); High Res. MS (neg. ion FAB): Calcd for $C_{69}H_{107}N_5O_{21}$ 1341.7458, measured mass: 1341.7463.

Anal. Calcd for $C_{69}H_{107}N_5O_{21} \cdot 0.75\ PhCH_3$: C, 63.17; H, 8.06; N, 4.96
Found: C, 62.83; H, 8.09; N, 5.00

The following representative compounds can be prepared from rapamycin and the appropriate terminally-N-substituted amino acid by employing the method used to prepare the title compound in Example 2.

Rapamycin-31,42-diester with N-[(fluorenylmethoxy)carbonyl]-alanylserine
Rapamycin-31,42-diester with N-[(fluorenylmethoxy)carbonyl]-glycylglycine
Rapamycin-31,42-diester with N-[(ethoxy)carbonyl]-arginylmethionine
Rapamycin-31,42-diester with N-[(4'-chlorophenoxy)carbonyl]-histidylarginine
Rapamycin-31,42-diester with N-[(phenoxy)carbonyl]-tryptophanylleucine
Rapamycin-31,42-diester with N-[(phenylmethoxy)carbonyl)]-N-methylglycyl-N-ethyl-alanine
Rapamycin-31,42-diester with N-[(phenylmethoxy)carbonyl]-N-methyl-ß-alanylphenyl- alanine
Rapamycin-31,42-diester with N-[(1,1-dimethylethoxy)carbonyl]-cysteinylglycine

Example 3

Rapamycin-31,42-diester with N-[(1,1-dimethylethoxy)carbonyl]-N-methylglycine

Under anhydrous conditions, an ice cold solution of rapamycin (2 g, 2.18 mmole) and N^{α}-Boc sarcosine (1.65 g, 8.75 mmole) in 20 ml of anhydrous dichloromethane was treated with dicyclohexylcarbodiimide (1.8 g, 8.7 mmole) followed by 4-dimethylaminopyridine (1 g, 8.7 mmole). After stirring overnight at ambient temperature, the precipitated solid was collected and washed with dichloromethane. The combined filtrates were evaporated to dryness to give an amorphous amber solid (3 g). The crude product was purified by flash chromatography (on silica Merck 60, elution with hexane-ethylacetate 1:1, v/v) to provide the title compound (0.75 g, 27.4%) along with the 42-monoester of Example 4. HPLC analysis showed that the diester is a 19.8:1 mixture of two conformers. The multiplicity of the NMR peaks suggests the presence of amide rotamers.

^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.411, 1.438, 1.448 and 1.474 (m, 18 H, $COOBu^t$), 2.91 (m, 6H, NCH_3), 3.14 (s, 3H, CH_3O), 3.34 (s, 3H, CH_3O), 3.37 (s, 3H, CH_3O), 4.73 (broad, 1H, *42*-CHO), 4.82 (2s, 1H, OH); High Res. MS (neg. ion FAB): Calcd. for $C_{67}H_{105}N_3O_{19}$ 1255.7342, measured mass 1255.7289.

Anal. Calcd for $C_{67}H_{105}N_3O_{19}$:	C, 64.04; H, 8.42; N, 3.34
Found:	C, 64.14; H, 8.74; N, 3.63

The following representative compounds can be prepared from rapamycin and the appropriate terminally-N-substituted amino acid by employing the method used to prepare the title compound in Example 3.

Rapamycin-31,42-diester with N-[(ethoxy)carbonyl]-tyrosine
Rapamycin-31,42-diester with N-[(fluorenylmethoxy)carbonyl]-phenylalanine
Rapamycin-31,42-diester with N-[(3',4',5'-trihydroxyphenoxy)carbonyl]-isoleucine
Rapamycin-31,42-diester with N-[(1,1-dimethylethoxy)carbonyl)-glutamine
Rapamycin-31,42-diester with N-[(phenoxy)carbonyl]-N-methylalanine
Rapamycin-31,42-diester with N-[(propyloxy)carbonyl]-4-aminobutryic acid
Rapamycin-31,42-diester with N-[(phenylmethoxy)carbonyl]-7-aminoheptanoic acid
Rapamycin-31,42-diester with N-[(fluorenylmethoxy)carbonyl]-serine

Example 4

Rapamycin-42-ester with N-[(1,1-dimethylethoxy)carbonyl]-N-methylglycine

Under anhydrous conditions, an ice cold solution of rapamycin (0.95 g, 1.02 mmole) and N^{α}-Boc sarcosine (0.21 g, 1.1 mmole) in 20 mL of anhydrous dichloromethane was treated with dicyclohexylcarbodiimide 0.21 g, 1 mmole) followed by 4-dimethylaminopyridine (0.12 g, 1 mmole). After stirring for 4 hours at ambient temperature, the precipitated solid was collected and washed with dichloromethane. The combined filtrates were concentrated *in vacuo* to give an amorphous amber solid. Flash chromatography of the crude product (on silica Merck 60, elution with hexane-ethylacetate 1:1 v/v to remove the diester of Example 3, followed by chloroform-ethylacetate-methanol 75:25:1 v/v) provided partially purified title compound (0.38 g, 35%). Pure product was obtained by preparative HPLC (Waters Prep 500, silica gel, chloroform-ethylacetate-methanol 75:25:1 v/v, flow rate 250 mL/min). HPLC analysis showed that the ester is a 6.6:1 mixture of two conformers. The multiplicity of NMR peaks suggests the presence of amide rotamers.

^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.42-1.46 (ds, 9H, $COOBu^t$), 2.91 (ds, 3H, NCH_3), 1.644 (s, 3H, CH_3C=C), 1.738 (s, 3H, CH_3C=C), 3.12 (s, 3H, CH_3O), 3.32 (s, 3H, CH_3O), 3.35 (s. 3H, CH_3O), 4.18 (d, 1H, CHOH), 4.71 (broad, 1H, *42*-CHO), 4.78 (broad s, 1H, OH); High Res. MS (neg. ion FAB): Calcd for $C_{59}H_{92}N_2O_{16}$ 1084.6446, measured mass 1084.6503.

Anal. Calcd for $C_{59}H_{92}N_2O_{16}$: C, 65.29; H, 8.54; N, 2.58
Found: C, 65.25; H, 8.52; N, 2.42

The following representative compounds can be prepared from rapamycin and the appropriate terminally-N-substituted amino acid by employing the method used to prepare the title compound in Example 4.

Rapamycin-42-ester with N-[(ethoxy)carbonyl]-tyrosine
Rapamycin-42-ester with N-[(fluorenylmethoxy)carbonyl]-phenylalanine
Rapamycin-42-ester with N-[(3',4',5'-trihydroxyphenoxy)carbonyl]-isoleucine
Rapamycin-42-ester with N-[(1,1-dimethylethoxy)carbonyl)-glutamine
Rapamycin-42-ester with N-[(phenoxy)carbonyl]-N-methylalanine
Rapamycin-42-ester with N-[(propyloxy)carbonyl]-4-aminobutyric acid
Rapamycin-42-ester with N-[(phenylmethoxy)carbonyl]-7-aminoheptanoic acid

Rapamycin-31,42-diester with N-[(fluorenylmethoxy)carbonyl]serine

Example 5

Rapamycin-31,42-diester with 5-(1,1-dimethylethoxy)-2-[[(1,1-dimethylethoxy)-carbonyl]amino]-5-oxopentanoic acid

Under anhydrous conditions, an ice cold solution of rapamycin (4 g, 4.37 mmole) and L-glutamic acid N^{α}-Boc-γ-tert-butylester (4.9 g, 16.1 mmole) in 40 mL of dry dichloromethane was treated with dicyclohexylcarbodiimide (1.8 g, 8.7 mmole) followed by 4-dimethylaminopyridine (1 g, 8.7 mmole). After stirring overnight at room temperature, the precipitated solid was collected and washed with dichloromethane. The combined filtrates were concentrated *in vacuo* to provide 11 g of an amorphous amber solid. The crude product was purified by flash chromatography (on silica Merck 60, gradient elution with hexane-ethylacetate from 2:1 to 1:1, v/v) to yield 4.52 g (69.6%) of the title compound along with the 42-monoester of Example 6. HPLC analysis showed that the diester consists of a 6.6:1 mixture of two conformers.

^1H NMR ($CDCl_3$, 400 MHz): δ 1.42 (m, 36 H, $COOBu^t$), 1.646 (s, 3H, $CH_3C{=}C$), 1.701 (s, 3H, $CH_3C{=}C$), 3.13 (s, 3H, CH_3O), 3.34 (s, 3H, CH_3O), 3.36 (s, 3H, CH_3O), 4.735 (m, 2H, OH+42-CH-O); High Res. MS (neg. ion FAB): calc. for $C_{79}H_{125}N_3O_{23}$ 1483.8715, measured mass 1483.8714.

Anal. Calcd for $C_{79}H_{125}N_3O_{23}$:	C, 63.90; H, 8.49; N, 2.83
Found:	C, 63.63; H, 8.41; N, 2.44

The following representative compounds can be prepared from rapamycin and the appropriately terminally-N-substituted amino diacid monoester by employing the method used to prepare the title compound in Example 5.

Rapamycin-31,42-diester with 6-(phenylmethoxy)-2-[[fluorenylmethoxy)carbonyl]-amino]-6-oxohexanoic acid

Rapamycin-31,42-diester with 6-(4'-methylphenoxy)-3-[[(phenylmethoxy)carbonyl]-amino-6-oxohexanoic acid

Rapamycin-31,42-diester with 6-(ethoxy)-4-[[(phenoxy)carbonyl]amino]-6-oxo-hexanoic acid

Rapamycin-31,42-diester with 6-(methoxy)-5-[[(ethoxy)carbonyl]amino]-6-oxo-hexanoic acid

Rapamycin-31,42-diester with 4-(phenoxy)-2-[N-[(1,1-dimethylethoxy)carbonyl]-N-methylamino]-4-oxobutanoic acid

Rapamycin-31,42-diester with 4-(phenylmethoxy)-3-[N-[(methoxy)carbonyl]-N-methylamino]-4-oxobutanoic acid

Example 6

Rapamycin-42-ester with 5-(1,1-dimethylethoxy)-2-[[(1,1-dimethylethoxy)-carbonyl]amino]-5-oxopentanoic acid

The title compound (1.14 g, 20.6%) was separated from the 31,42-diester as described in Example 5 and isolated as the quarter hydrate/mono-ethyl acetate solvate. HPLC analysis showed that the monoester is a 11.5:1 mixture of two conformers.

^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.425 (m, 18H, $COOBu^t$), 1.643 (s, 3H, $CH_3C{=}C$), 1.737 (s, 3H, $CH_3C{=}C$), 3.13 (s, 3H, CH_3O), 3.32 (s, 3H, CH_3O), 3.36 (s, 3H, CH_3O), 4.17 (d, 1H, C*H*OH), 4.71 (M, 1H, *42*-CHO), 4.785 (s, 1H, OH); High Resolution MS (neg. ion FAB): Calc. for $C_{65}H_{102}N_2O_{18}$ 1198.7127, measured mass 1198.7077.

Anal. Calcd for $C_{65}H_{102}N_2O_{18} \cdot CH_3COOEt \cdot 0.25\ H_2O$:

	C, 64.13, H, 8.60; N, 2.17
Found:	C, 64.18; H, 8.52; N, 2.01

The following representative compounds can be prepared from rapamycin and the appropriately terminally-N-substituted amino diacid monoester by employing the method used to prepare the title compound in Example 6.

Rapamycin-42-ester with 6-(phenylmethoxy)-2-[[fluorenylmethoxy)carbonyl]-amino]-6-oxohexanoic acid

Rapamycin-42-ester with 6-(4'-methylphenoxy)-3-[[(phenylmethoxy)carbonyl]-amino-6-oxohexanoic acid

Rapamycin-42-iester with 6-(ethoxy)-4-[[(phenoxy)carbonyl]amino]-6-oxo- hexanoic acid

Rapamycin-42-ester with 6-(methoxy)-5-[[(ethoxy)carbonyl]amino]-6-oxo- hexanoic acid

Rapamycin-42-ester with 4-(phenoxy)-2-[N-[(1,1-dimethylethoxy)carbonyl]-N-methylamino]-4-oxobutanoic acid

Rapamycin-42-ester with 4-(phenylmethoxy)-3-[N-[(methoxy)carbonyl]-N-methylamino]-4-oxobutanoic acid

Example 7

Rapamycin-31,42-diester with 2-[[(1,1-dimethylethoxy)carbonyl]amino]-4-oxo-4-(phenylmethoxy) butanoic acid

Under anhydrous conditions, 295mg (1.21mmol) of 2,4,6 trichlorobenzoyl chloride was added to a solution of 391mg(1.21mmol) of N^{α}-Boc-L-aspartic acid-β-benzyl ester and 170μL (1.21mmol) of Et_3N in 1 mL of THF at room temperature. After stirring for 30 minutes, 500 mg (0.55mmol) of rapamycin and 295 mg (2.42 mmol) of dimethylaminopyridine was added and the reaction was left to stir overnight. The reaction mixture was then filtered and the filtrate concentrated *in vacuo*. Pure product (200 mg, 25%) was obtained by preparative HPLC (5 cm column, 40 % ethyl acetate-hexane). The product was isolated as the heptahydrate.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.347 (s, 10 H, Ar), 6.223, 5.126 (s, 4 H, CH_2Ph), 4.698 (m, 1 H, CH-CO_2), 4.587 (m, 2 H, NH), 3.353 (s, 3 H, CH_3O), 3.337 (s, 3 H, CH_3O), 3.301 (s, 3 H, CH_3O), 2.775 (m, 4 H, CH_2CO_2); IR (KBr) 3420 (OH), 2935 (CH), 2920 (CH), 1730 (C=O), 1650, 1500, 1455, 1370, 1170 cm^{-1}; MS (neg. ion FAB) 1523 (M^-), 1433, 297, 248, 205, 148, 44, 25 (100).

Anal. Calcd for $C_{83}H_{117}N_3O_{23}\cdot 7H_2O$ '	C, 60.40; H, 7.09; N, 2.54
Found:	C, 60.54; H, 7.28; N, 2.56

Example 8

Rapamycin-31,42-diester with 3-[[(1,1-dimethylethoxy)carbonyl]amino]-4-oxo-4-(phenylmethoxy) butanoic acid

Under anhydrous conditions, 532 mg (2.18 mmol) of 2,4,6 trichlorobenzoyl chloride in 1 mL THF was added to a solution of 704 mg (2.18 mmol) of N^{α}-Boc-L-aspartic acid-α-benzyl ester and 303 μL (2.18 mmol) of Et_3N in 5 mL of THF at room temperature. After stirring for 20 minutes, the reaction mixture was filtered over sintered glass, and the precipitate was washed with THF. The filtrate was concentrated *in vacuo* to give a thick oil. The oil was dissolved in 5 mL of benzene and 1.00 g (1.09 mmol) of rapamycin and 532 mg (4.36 mmol) of dimethylaminopyridine in 1 mL of benzene was added dropwise. The reaction was stirred for 2 hr, poured into ethyl acetate, and washed consecutively with 0.5 N HCl and brine. The solution was dried over sodium sulfate, decanted, concentrated *in vacuo* to give a white foamy solid, which was purified via flash chromatography on a 60 mm x 100 mm silica column (20-40 % ethyl acetate/hexane as eluant) to give 532 mg (33 %) of the title compound which was isolated as the hydrate.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.362 (s, 10 H, *Ar*), 5.193 (s, 4 H, CH_2Ph), 4.596 (m, 1 H, *CH*-CO_2), 4.586 (m, 2 H, *NH*), 3.336 (s, 3 H, CH_3O), 3.306 (s, 3 H, CH_3O), 3.145 (s, 3 H, CH_3O); IR (KBr) 3410 (OH), 2950 (CH), 2920 (CH), 1735 (C=O), 1710 (C=O), 1640, 1490, 1445, 1350, 1150 cm $^{-1}$; MS (neg. ion FAB) 1524 (M^-), 1434, 297, 248, 232, 214, 205, 167, 148, 42 (100), 26.

Anal. Calcd for $C_{83}H_{117}N_3O_{23} \cdot H_2O$: C, 65.38; H, 7.73; N, 2.76
Found: C, 64.85; H, 7.67; N, 2.56

Example 9

Rapamycin-42-ester with 3-[[(1,1-dimethylethoxy)carbonyl]amino]-4-oxo-4-(phenylmethoxy) butanoic acid

The title compound (374 mg, 23%) was prepared by the method described in the previous Example and separated from the compound described in the previous Example by flash chromatography (20-40% ethyl acetate/hexane as the eluant) and isolated as the sesquihydrate.

^1H NMR ($CDCl_3$, 400 MHz) δ 7.356 (s, 5 H, *Ar*), 5.185 (s, 2 H, CH_2Ph), 4.635 (m, 1 H, CH-CO_2), 4.582 (m, 1 H, *NH*), 3.330 (s, 6 H, CH_3O), 3.135 (s, 3 H, CH_3O); IR (KBr) 3410 (OH), 2950 (CH), 2920 (CH), 1735 (C=O), 1710 (C=O), 1640, 1490, 1445, 1350, 1150 cm $^{-1}$; MS (neg. ion FAB) 1213 (M^-), 1127, 590, 168, 42, 25, 17 (100).

Anal. Calcd for $C_{67}H_{98}N_2O_{18} \cdot 1.5\ H_2O$:	C, 63.64; H, 8.21; N, 2.22
Found:	C, 63.64; H, 7.51; N, 2.13

Example 10

Rapamycin-42-ester with 5-(1,1-dimethyloxy)-4-[[(1,1-dimethylethoxy)carbonyl] amino]-5-oxopentanoic acid

Under anhydrous conditions, an ice cold solution of rapamycin (4 g, 4.37 mmole) and L-glutamic acid N^{α}-Boc-α-tert-butylester (4.9 g, 16.1 mmole) in 40 mL of anhydrous dichloromethane was treated with dicyclohexylcarbodiimide (1.8 g, 8.7 mmole) followed by 4-dimethylamino pyridine (1 g, 8.7 mmole). After stirring overnight at ambient temperature, the precipitated solid was collected and washed with dichloromethane. The combined filtrates were concentrated *in vacuo* to give 9 g of an amorphous amber solid. The crude product was purified by flash chromatography (on silica Merck 60, gradient elution with hexane-ethylacetate from 2:1 to 3:2, v/v) to provide 1.35 g (25.7%) of the title compound along with the 31,42-diester of Example 11. HPLC analysis showed that the monoester is a 7.5 :1 mixture of two conformers.

^1H NMR ($CDCl_3$, 400 MHz): δ 1.43 (s, 9H, $COOBu^t$) and 1.46 (s, 9H, $COOBu^t$), 1.65 (s, 3H, CH_3C=C), 1.75 (s, 3H, CH_3C=C), 3.14 (s, 3H, CH_3O), 3.34 (s, 3H, CH_3O), 3.38 (s, 3H, CH_3O), 4.18 (d, 1H, CH-OH), 4.65 (m, 1H, 42-CHO), 4.80 (s, 1H, OH);
High Res. MS (neg. ion FAB): Calc. for $C_{65}H_{102}N_2O_{18}$: 1198.7126, measured mass 1198.7135.

Anal. Calcd for $C_{65}H_{102}N_2O_{18}$:	C, 65.09; H, 8.57; N, 2.34
Found	C, 65.04; H, 8.33; N, 2.64

Example 11

Rapamycin-31,42-diester with 5-(1,1-dimethylethoxy)-4-[[(1,1-dimethylethoxy)-carbonyl]- amino]-5-oxopentanoic acid

The title compound was prepared (0.83 g, 12.8%) along with the 42-monoester as described in Example 10. HPLC analysis showed that the diester is a 7.7:1 mixture of two conformers.

^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.43 (s, 18H, $COOBu^t$), 1.46 (s, 18H, $COOBu^t$), 1.659 (s, 3H, CH_3C=C), 1.759 (s, 3H, CH_3C=C), 3.14 (s, 3H, CH_3O), 3.34 (s, 3H, CH_3O), 3.38 (s, 3H, CH_3O), 4.66 (m, 1H, *42*-CHO), 4.72 (s, 1H, OH); High Res. MS (neg. ion FAB): Calcd for $C_{79}H_{125}N_3O_{23}$: 1483.8704, measured mass 1483.8636.

Anal. Calcd for $C_{79}H_{125}N_3O_{23}$:	C, 63.90; H, 8.49; N, 2.83
Found:	C, 63.68; H, 8.60; N, 3.20

Example 12

Rapamycin-42-ester with N^{α}, N^{ε}-bis[(1,1-dimethylethoxy)carbonyl]-L-lysine

Under anhydrous conditions, a solution of rapamycin (3 g, 3.28 mmole) and N^{α}, N^{ε}-bis-Boc-L-lysine (4.5 g, 13 mmole) in 40 mL of anhydrous dichloromethane was treated with dicyclohexylcarbodiimide (1.35 g, 6.56 mmole) followed by 4-dimethylaminopyridine (0.8 g, 6.56 m mole). After stirring overnight at ambient temperature, the precipitated solid was collected and washed with dichloromethane. The combined filtrates were concentrated *in vacuo* to give an amorphous amber solid. Flash chromatography of the crude product (on silica Merck 60, elution with hexane-ethylacetate 1:1 v/v) gave partially purified title compound. Pure product (0.8 g, 19.6%) was obtained by preparative HPLC (Waters Prep 500, silica gel, hexane-ethylacetate 3:2 v/v, flow rate 250 mL/min). HPLC analysis showed that the monoester is a 9:1 mixture of two conformers.

^1H NMR ($CDCl_3$, 400 MHz): δ 1.438 (m, 9H, $COOBu^t$), 1.455 (s, 9H, $COOBu^t$), 1.652 (s, 3H, CH_3C=C), 1.752 (s, 3H, CH_3C=C), 3.14 (s, 3H, CH_3O), 3.33(s, 3H, CH_3O), 3.37 (s, 3H, CH_3O), 4.18 (d, 1H, CHOH), 4.72 (m, 1H, 42-CHO), 4.79 (s, 1H, OH); High Res. MS (neg. ion FAB): Calcd for $C_{67}H_{107}N_3O_{18}$: 1241.7549, measured mass 1241.7604.

Anal. Calcd for $C_{67}H_{107}N_3O_{18}$:	C, 64.76; H, 8.68; N, 3.38
Found:	C, 64.58; H, 9.01; N, 3.10

Example 13

Rapamycin-31,42-diester with N^{α}, N^{ε}-bis[(1,1-dimethylethoxy)carbonyl]-L-lysine

Under a nitrogen atmosphere, a solution of N^{α},N^{ε} bis-Boc-L-lysine (1.038 g, 3 mmole) and triethylamine (0.42 mL, 3 mmmole) in 10 mL of anhydrous THF was treated in one portion with 2,4,6-trichlorobenzoyl chloride (0.73 g, 3 mmole). After stirring for 20 minutes at ambient temperature, the precipitated solid was collected and the filtrate was concentrated *in vacuo* . The resulting mixed anhydride was dissolved in 5 mL of benzene and added to a stirred solution of rapamycin (1 g, 1.09 mmole) containing 4-dimethylamino pyridine (0.59 g, 4.8 mmole) in 10 mL of benzene. After stirring at ambient temperature overnight, the precipitated solid was collected and the filtrate was evaporated to dryness (yellow foam). The crude product was purified by flash chromatography (on silica Merck 60, elution with hexane-ethylacetate 1:1) to provide title compound (1.15 g, 67%). HPLC analysis shows that the diester is a 9:1 mixture of two conformers.

^1H NMR ($CDCl_3$, 400 MHz): δ 1.426 (m, 9H, $COOBu^t$), 1.438 (s, 9H, $COOBu^t$), 1.443 (s, 9H, $COOBu^t$), 1.446 (s, 9H, $COOBu^t$), 3.141 (s, 3H, CH_3O), 3.36 (s, 3H, CH_3O), 3.378 (s, 3H, CH_3O), 4.68-4.76 (m, 2H, OH and 42-CHO); High res. MS (neg. ion FAB): Calcd. for $C_{83}H_{135}N_5O_{23}$ 1569.9526, measured mass 1569.9537.

Anal. Calcd. for $C_{83}H_{135}N_5O_{23}$:	C, 63.46; H, 8.66; N, 4.46
Found:	C, 63.06; H, 8.34; N, 4.09

Example 14.

Rapamycin-14,31,42-tris(monobenzylsuccinate)

To a solution of 5.0 g (5.47 mmol) of rapamycin, 3.41 g (16.41 mmol) of monobenzylsuccinate, and 3.15 g (16.41 mmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride in 20 mL of dry dichloromethane was added 200 mg of 4-dimethylaminopyridine. The solution was stirred at room temperature for 3 days. The reaction mixture was poured into 2 N HCl and extracted three times with ethyl acetate. The organic layers were combined, washed with brine, dried over anhydrous sodium sulfate, decanted, and concentrated in vacuo to give a light yellow foam. Flash chromatography on a 60 mm x 150 mm silica gel column eluting with 20 % ethyl acetate/hexane to 75 % ethyl acetate/hexane gave three fractions. Fraction #1, upon concentration, gave 330 mg (4.1 %) of pure rapamycin-14,31,42-tris-(monobenzylsuccinate).

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.353 (bs, 15 H, *arom*), 5.168 (d, J = 2.0 Hz, 1 H, CH-O_2C), 5.148 (m, 6 H, CH_2Ph), 4.672 (m, 1 H, CO_2CH-CHOMe), 3.355 (s, 3 H, CH_3O-), 3.337 (s, 3 H, CH_3O-), 3.327 (s, 3 H, CH_3O-), 2.697 (m, 12 H, $O_2CCH_2CH_2CO_2CH_2$Ph), 1.745 (s, 3 H, CH_3C=C), 1.655 (s, 3 H, CH_3C=C); IR (KBr) 3450 (OH), 2950 (CH), 1745 (C=O), 1650, 1460, 1385, 1360, 1160, 1105, 995 cm^{-1}.

Analysis Calcd for $C_{84}H_{109}NO_{21} \cdot 3\,H_20$	C 66.27;	H 7.56;	N 0.92
Found	C 65.96;	H 7.24;	N 1.00

The following representative compounds can be prepared from rapamycin and the appropriate half acid-ester by employing the method used to prepare the title compound in Example 14.

Rapamycin-14,31,42-tris (monomethylsuccinate)
Rapamycin-14,31,42-tris (monophenyl-3',3'-dimethylglutarate)
Rapamycin-14,31,42-tris (mono t-butyl-3'-methylglutarate)
Rapamycin-14,31,42-tris (monobenzylthiodiglycolate)
Rapamycin-14,31,42-tris (monohexyldiglycolate)
Rapamycin-14,31,42-tris (monopropylphthalate)
Rapamycin-14,31,42-tris (monoethyl-2',6'-pyridinedicarboxylate)

Example 15.

Rapamycin-31,42-bis(monobenzylsuccinate)

Fraction # 2, obtained from the procedure employed in Example 14, gave 1.25 g (17.7 %) of pure rapamycin-31,42-bis(monobenzylsuccinate) upon concentration.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.351 (bs, 10 H, *arom*), 5.168 (d, J = 2.0 Hz, 1 H, CH-O_2C), 5.125 (m, 4 H, CH_2Ph), 4.680 (m, 1 H, CO_2CH-CHOMe), 3.356 (s, 3 H, CH_3O-), 3.329 (s, 3 H, CH_3O-), 3.146 (s, 3 H, CH_3O-), 2.639 (m, 8 H, $O_2CCH_2CH_2CO_2CH_2$Ph), 1.748 (s, 3 H, CH_3C=C), 1.654 (s, 3 H, CH_3C=C); IR (KBr) 3450 (OH), 2940 (CH), 1740 (C=O), 1650, 1455, 1380, 1355, 1160, 1105, 995 cm^{-1}; MS (neg. ion FAB) 1294 (M-), 1202, 1103, 1012, 590, 511, 475, 297, 207, 167, 148, 99 (100); High Res. MS (neg. ion FAB) Calcd for $C_{73}H_{99}NO_{19}$ 1293.68108, found 1293.6811.

Analysis Calcd for $C_{73}H_{99}NO_{19} \cdot H_20$	C 66.82;	H 7.70;	N 1.07
Found	C 67.17;	H 7.67;	N 1.23

The following representative compounds can be prepared from rapamycin and the appropriate half acid-ester by employing the method used to prepare the title compound in Example 15.

Rapamycin-31,42-bis (monomethylsuccinate)
Rapamycin-31,42-bis (monophenyl-3',3'-dimethylglutarate)
Rapamycin-31,42-bis (mono t-butyl-3'-methylglutarate)
Rapamycin-31,42-bis (monobenzylthiodiglycolate)
Rapamycin-31,42-bis (monohexyldiglycolate)
Rapamycin-31,42-bis (monopropylphthalate)
Rapamycin-31,42-bis (monoethyl-2',6'-pyridinedicarboxylate)

Example 16.

Rapamycin-42-(monobenzylsuccinate)

Fraction # 3, obtained from the procedure employed in Example 14, gave 930 mg (15.4 %) of pure rapamycin-42-monobenzylsuccinate upon concentration.

^1H NMR ($CDCl_3$, 400 MHz) δ 7.355 (bs, 5 H, *arom*), 5.141 (m, 2 H, CH_2Ph), 4.680 (m, 1 H, CO_2CH-CHOMe), 3.364 (s, 3 H, CH_3O-), 3.333 (s, 3 H, CH_3O-), 3.141 (s, 3 H, CH_3O-), 2.698 (m, 4 H, $O_2CCH_2CH_2CO_2CH_2Ph$), 1.751 (s, 3 H, $CH_3C{=}C$), 1.655 (s, 3 H, $CH_3C{=}C$); IR (KBr) 3450 (OH), 2940 (CH), 1740 (C=O), 1645, 1455, 1380, 1165, 1105, 990 cm^{-1}; MS (neg. ion FAB) 1103 (M-), 1045, 1012, 624, 590, 167, 99 (100); High Res. MS (neg. ion FAB) Calcd for $C_{62}H_{89}NO_{16}$ 1103.6181, found 1103.6048.

Analysis Calcd for $C_{62}H_{89}NO_{16} \cdot H_2O$	C 66.36;	H 8.02;	N 1.24
Found	C 66.02;	H 7.69;	N 1.26

The following representative compounds can be prepared from rapamycin and the appropriate half acid-ester by employing the method used to prepare the title compound in Example 16.

Rapamycin-42-(monomethylsuccinate)
Rapamycin-42-monophenyl-3',3'-dimethylglutarate)
Rapamycin-42-(mono t-butyl-3'-methylglutarate)
Rapamycin-42-(monobenzylthiodiglycolate)
Rapamycin-42-(monohexyldiglycolate)
Rapamycin-42-(monopropylphthalate)
Rapamycin-42-(monoethyl-2',6'-pyridinedicarboxylate)

Example 17.

Rapamycin-31,42-bishemiglutarate

To a solution of 2.0 g (2.2 mmol) of rapamycin in 10 mL of dry dichloromethane was added 1.24 g (10.9 mmol) of glutaric anhydride followed by 881 uL (861 mg, 10.9 mmol) of pyridine. To this was added 200 mg of 4-dimethylaminopyridine and the reaction mixture was allowed to reflux for 8 h. The solution was cooled to room temperature, poured into 2 N HCl, and extracted three times with dichloromethane. The combined organic extracts were washed with brine, dried over anhydrous sodium sulfate, decanted, and concentrated in vacuo to give a yellow foam. The crude product was purified via reverse phase HPLC on a C_{18} column eluting starting with 60 % acetonitrile/water. Collected, after, concentration, 586 mg (24 %) of rapamycin-31,42-bishemiglutarate.

^1H NMR ($CDCl_3$, 400 MHz) δ 5.398 (m, 1 H, $-CO_2CHCHOMe$), 4.683 (m, 1 H, $-CO_2CHCHOMe$), 3.364 (s, 3 H, CH_3O-), 3.362 (s, 3 H, CH_3O-), 3.106 (s, 3 H, CH_3O-), 2.407 (m, 8 H, $-O_2CCH_2CH_2CH_2CO_2H$), 1.960 (m, 4 H, $-O_2CCH_2CH_2CH_2CO_2H$), 1.770 (s, 3 H, $CH_3C=C$), 1.653 (s, 3 H, $CH_3C=C$); ^{13}C NMR ($CDCl_3$, MHz) 211.45 (C=O), 206.84 (C=O), 200.44 (C=O), 177.83 (C=O), 177.04 (C=O), 172.43 (C=O), 171.20 (C=O), 165.27 (C=O), 159.08 (C=O); IR (KBr) 3430 (OH), 2940 (CH), 2880 (CH), 1745 (C=O), 1685, 1625, 1580, 1450, 1385, 1330, 1200, 1140, 1100, 990 cm^{-1}; MS (neg. ion FAB) 1140 (M-H), 1122, 1026, 990, 946, 913, 590, 475, 435, 321, 167, 148, 131 (100), 113; High Res. MS (neg. ion FAB) Calcd for $C_{61}H_{90}O_{19}N$ (M-H) 1140.6107, Found 1140.6106.

Analysis Calcd for $C_{61}H_{91}O_{19}N \cdot H_2O$	C 63.15;	H 8.02;	N 1.20
Found	C 63.35;	H 7.88;	N 1.40

The following representative compounds can be prepared from rapamycin and the appropriate anhydride by employing the method used to prepare the title compound in Example 17.

Rapamycin-31,42-bishemi-3'-methylglutarate
Rapamycin-31,42-bishemi-3',3'-dimethylglutarate
Rapamycin-31,42-bishemi-3'-oxoglutarate
Rapamycin-31,42-bishemi-3'-thioglutarate
Rapamycin-31,42-bishemi-phthalate
Rapamycin-31,42-bishemi-2',3'-pyridine dicarboxylate

Example 18.

Rapamycin-31,42-hemiglutarate bissodium salt

Purified bis-31,42-hemiglutarate of rapamycin (740 mg, 649 umol), prepared as described in Example 17, was dissolved in 5 mL of 95 % ethanol and 107 mg (1.27 mmol) of sodium bicarbonate was added. Water (1 mL) was added to completely dissolve the salt. Once dissolved, the light yellow solution was concentrated in vacuo to give a foamy yellow solid. The foam was dried in a drying pistol for 24 h, refluxing over acetone at reduced pressure to give 520 mg of the bissodium salt.

^1H NMR (d_6-DMSO, 400 MHz) δ 5.235 (m, 1 H, -CHO_2C), 4.498 (m, 1 H, $MeOCHCHO_2C$-), 3.287 (s, 6 H, 2 CH_3O-), 3.235 (s, 3 H, CH_3O-), 2.245 (m, 8 H, $O_2CCH_2CH_2CH_2CO_2^-$), 1.712 (s, 3 H, CH_3C=C), 1.593 (s, 3 H, CH_3C=C); IR (KBr) 3420 (OH), 2920 (CH), 1725 (C=O), 1675, 1620, 1560, 1450, 1400, 1375, 1230, 1195, 1130, 1090, 980 cm^{-1}; MS (neg. ion FAB) 1112 (M-1, free acid), 994, 589, 475, 297, 167, 148, 117, 99 (100); High Res. MS (neg. ion FAB) Calcd for $C_{61}H_{89}O_{19}NNa$ (M-Na) 1162.5926, Found 1162.5899.

Analysis Calcd for $C_{61}H_{89}O_{19}NNa_2 \cdot H_2O$	C 60.85;	H 7.56;	N 1.16
Found	C 60.67;	H 7.36;	N 1.58

Example 19.

Rapamycin-31,42-bishemiglutarate bistromethamine salt

Purified bis-31,42 hemiglutarate of rapamycin (950 mg, 833 umol), prepared as described in Example 17, was dissolved in 5 mL of 95 % ethanol and 197 mg (1.63 mmol) of tris(hydroxymethyl)methylamine was added. Water (1 mL) was added to completely dissolve the amine. Once dissolved, the yellow solution was concentrated in vacuo to give a foamy yellow solid. The very hygroscopic foam was dried in a drying pistol for 24 h, refluxing over acetone at reduced pressure to give 900 mg (78 %) of the bistromethamine salt.

^1H NMR (d_6-DMSO, 400 MHz) δ 5.253 (m, 1 H, -CHO_2C), 4.523 (m, 1 H, $MeOCHCHO_2C$-), 3.347 (s, 6 H, 2 CH_3O-), 3.276 (s, 3 H, CH_3O-), 2.289 (m, 8 H, $O_2CCH_2CH_2CH_2CO_2^-$), 1.681 (s, 3 H, CH_3C=C), 1.595 (s, 3 H, CH_3C=C); IR (KBr) 3400 (OH), 2920 (CH), 1730 (C=O), 1620, 1555, 1450, 1400, 1370, 1185, 1060, 980 cm^{-1}; MS (neg. ion FAB) 1140 (M-H, free acid), 1028, 167, 148, 131 (100), 113; High Res. MS (neg. ion FAB) Calcd for $C_{61}H_{90}O_{19}N$ (M-H, free acid) 1140.6107, Found 1140.6069.

Analysis Calcd for $C_{69}H_{103}O_{25}N_3 \cdot 2\,H_2O$	C 58.77;	H 7.58;	N 2.98
Found	C 58.47;	H 7.94;	N 3.58

Example 20.

Rapamycin-42-hemi-3'-oxoglutarate

To a solution of 3.0 g (3.3 mmol) of rapamycin in 20 mL of dry dichloromethane was added 1.90 g (16.4 mmol) of diglycolic anhydride followed by 1.32 mL (1.29 g, 16.4 mmol) of pyridine. To this was added 200 mg of 4-dimethylaminopyridine and the reaction mixture was allowed to stir at room temperature for 2 days. The solution was cooled to room temperature, poured into 2 N HCl, and extracted three times with dichloromethane. The combined organic extracts were washed with brine, dried over anhydrous sodium sulfate, decanted, and concentrated in vacuo to give a yellow foam. The crude product was purified via reverse phase HPLC on a C_{18} column eluting starting with 60 % acetonitrile/water. After concentration, 870 mg (26 %) of rapamycin-42-hemi-3'-oxoglutarate and 500 mg (13 %) of rapamycin-31,42-bishemi-3'oxoglutarate were isolated.

^{1}H NMR (CDCl3, 400 MHz) δ 4.768 (m, 1 H, CO_2CH-CHOMe), 4.250 (m, 4 H, $O_2CCH_2OCH_2CO_2$), 3.356 (s, 3 H, CH_3O-), 3.331 (s, 3 H, CH_3O-), 3.139 (s, 3 H, CH_3O-), 1.759 (s, 3 H, CH_3C=C), 1.653 (s, 3 H, CH_3C=C);
IR (KBr) 3420 (OH), 2920 (CH), 2875 (CH), 1740 (C=O), 1720 (C=O), 1640, 1625, 1445, 1370, 1320, 1200, 1135, 1095, 980 cm^{-1}; MS (neg. ion FAB) 1028 (M - H), 327, 167 (100), 148, 133, 115; High Res. MS (neg. ion FAB) Calcd for $C_{55}H_{82}O_{17}N$ (M - H) 1028.5597, Found 1028.5599.

Analysis Calcd for $C_{55}H_{83}O_{17}N \cdot 3\ H_2O$	C 60.97;	H 8.22;	N 1.29
Found	C 61.33;	H 7.74;	N 1.69

The following representative compounds can be prepared from rapamycin and the appropriate half acid-ester by employing the method used to prepare the title compound in Example 20.

Rapamycin-42-hemi-3'-methylglutarate
Rapamycin-42-hemi-3',3'-dimethylglutarate
Rapamycin-42-hemi-3'-thioglutarate
Rapamycin-42-hemi-phthalate
Rapamycin-42-hemi-2',3'-pyridine dicarboxylate

Example 21.

Rapamycin-31,42-bishemi-3'-oxoglutarate

To a solution of 5.0 g (5.47 mmol) of rapamycin in 20 mL of dry dichloromethane was added 3.17 g (27.3 mmol) of diglycolic anhydride followed by 2.17 mL (2.12 g, 27.3 mmol) of pyridine. To this was added 400 mg of 4-dimethylaminopyridine and the reaction mixture was allowed to stir at reflux for 24 h. The solution was cooled to room temperature, poured into 2 N HCl, and extracted three times with dichloromethane. The combined organic extracts were washed with brine, dried over anhydrous sodium sulfate, decanted, and concentrated in vacuo to give a yellow foam. The crude product was purified via reverse phase HPLC on a C_{18} column eluting starting with 60 % acetonitrile/water. After concentration, 1.75 g (28 %) of rapamycin-31,42-bishemi-3'-oxoglutarate was isolated.

^{1}H NMR (CDCl3, 400 MHz) δ 4.785 (m, 1 H, $CO_2CHCHOMe$), 4.260 (m, 8 H, $O_2CCH_2OCH_2CO_2$), 3.360 (s, 3 H, CH_3O-), 3.343 (s, 3 H, CH_3O-), 3.143 (s, 3 H, CH_3O-), 1.775 (s, 3 H, CH_3C=C), 1.656 (s, 3 H, CH_3C=C); ^{13}C NMR ($CDCl_3$, MHz) 211.12 (C=O), 207.73 (C=O), 193.11 (C=O), 171.90 (C=O), 171.59 (C=O), 170.15 (C=O), 169.35 (C=O), 168.83 (C=O), 166.63 (C=O); IR (KBr) 3420 (OH), 2920 (CH), 2850 (CH), 1740 (C=O), 1645, 1625, 1440, 1370, 1190, 11300, 980 cm^{-1}; MS (neg. ion FAB) 1140 (M-H), 1122, 1026, 990, 946, 913, 590, 475, 435, 321, 167, 148, 131 (100), 113; High Res. MS (neg. ion FAB) Calcd for $C_{59}H_{86}O_{21}N$ (M - H) 1144.5701, Found 1144.5702.

Analysis Calcd for $C_{59}H_{87}O_{21}N$	C 61.82;	H 7.65;	N 1.22
Found	C 61.59;	H 7.36;	N 1.84

Example 22.

Rapamycin-31,42-bishemi-3'-oxoglutarate disodium salt

Purified bis-31,42 hemi-3'-oxoglutarate of rapamycin (720 mg, 629 umol) , prepared by the procedure employed in Example 21, was dissolved in 10 mL of 95 % ethanol and 106 mg (1.26 mmol) of sodium bicarbonate was added. Water (1 mL) was added to completely dissolve the salt. Once dissolved, the light yellow solution was concentrated in vacuo to give a foamy yellow solid. The foam was dried in a drying

pistol for 48 h, refluxing over dichloromethane at reduced pressure to give 435 mg (58 %) of the disodium salt.

^{1}H NMR (d_6-DMSO, 400 MHz) δ 4.975 (m, 1 H, -CHO_2C), 4.593 (m, 1 H, MeOCHCHO_2C-), 4.135 (s, 2 H, $^-O_2CCH_2OCH_2CO_2R$), 3.617 (s,2 H, $^-O_2CCH_2OCH_2CO_2R$), 3.299 (s, 6 H, 2 CH_3O-), 3.232 (s, 3 H, CH_3O-), 1.614 (s, 3 H, CH_3C=C), 1.553 (s, 3 H, CH_3C=C); IR (KBr) 3420 (OH), 2920 (CH), 1735 (C=O), 1615, 1445, 1395, 1380, 1320, 1220, 1130, 1090, 980 cm^{-1};
MS (neg. ion FAB) 1188 (M-1), 1166 (M-Na), 1144, 1051, 1028, 590, 459, 167, 155 (100), 148, 133, 115.

Analysis Calcd for $C_{59}H_{85}O_{21}NNa_2 \cdot 2H_2O$	C 57.79;	H 7.26;	N 1.14
Found	C 57.94;	H 7.11;	N 1.26

Example 23.

Rapamycin-31,42-bishemi-3'-oxoglutarate bistromethamine salt

Purified bis-31,42 hemi-3'-oxoglutarate of rapamycin (1.01 g, 882 umol), prepared by the procedure employed in Example 21, was dissolved in 10 mL of 95 % ethanol and 213 mg (1.76 mmol) of tris(hydroxymethyl)- methylamine was added. Water (1 mL) was added to completely dissolve the amine. Once dissolved, the yellow solution was concentrated in vacuo to give a foamy yellow solid. The very hygroscopic foam was dried in a drying pistol for 48 h, refluxing over dichloromethane at reduced pressure to give 805 mg (66 %) of the bistromethamine salt.

^{1}H NMR (d_6-DMSO, 400 MHz) δ 4.955 (m, 1 H, -CHO_2C), 4.600 (m, 1 H, MeOCHCHO_2C-), 4.149 (s, 2 H, $^-O_2CCH_2OCH_2CO_2R$), 3.770 (s, 2 H, $^-O_2CCH_2OCH_2CO_2R$), 3.407 (s, 6 H, 2 CH_3O-), 3.257 (s, 3 H, CH_3O-), 1.806 (s, 3 H, CH_3C=C), 1.614 (s, 3 H, CH_3C=C); IR (KBr) 3400 (OH), 2920 (CH), 1730 (C=O), 1620, 1550, 1450, 1395, 1370, 1200, 1060, 985 cm^{-1};
MS (neg. ion FAB) 1144 (M-H, free acid), 1028, 167, 148, 133 (100), 115.

Analysis Calcd for $C_{67}H_{109}O_{27}N_3 \cdot H_2O$	C 57.22;	H 7.90;	N 2.98
Found	C 57.26;	H 7.90;	N 3.15

Example 24.

Rapamycin-31,42-bishemisuccinate.

To a solution of 2.0 g (2.2 mmol) of rapamycin in 10 mL of dry dichloromethane was added 1.19 g (10.9 mmol) of succinic anhydride followed by 881 uL (861 mg, 10.9 mmol) of pyridine. To this was added 200 mg of 4-dimethylaminopyridine and the reaction mixture refluxed for 24 h. The solution was cooled to room temperature, poured into 2 N HCl, and extracted three times with dichloromethane. The combined organic extracts were washed with brine, dried over anhydrous sodium sulfate, decanted, and concentrated in vacuo to give a yellow foam. The crude product was purified via reverse phase HPLC on a C_{18} column gradient eluting starting with 20 % acetonitrile/water to 60 % acetonitrile/water. Collected, after, concentration, 770 mg (31 %) of rapamycin-31,42-bishemisuccinate.

The purified bis-31,42 hemisuccinate of rapamycin (770 mg, 686 umol) was dissolved in 10 mL of 95 % ethanol and 166 mg (1.37 mmol) of tris(hydroxymethyl)-methylamine was added. Water (1 mL) was added to completely dissolve the amine. Once dissolved, the yellow solution was concentrated in vacuo to give a foamy yellow solid. The very hygroscopic foam was dried in a drying pistol for 24 h, refluxing over acetone at reduced pressure to give 890 mg (95 %) of the bistromethamine salt. The bistromethane salt was evaluated in the standard pharmacological test procedures.

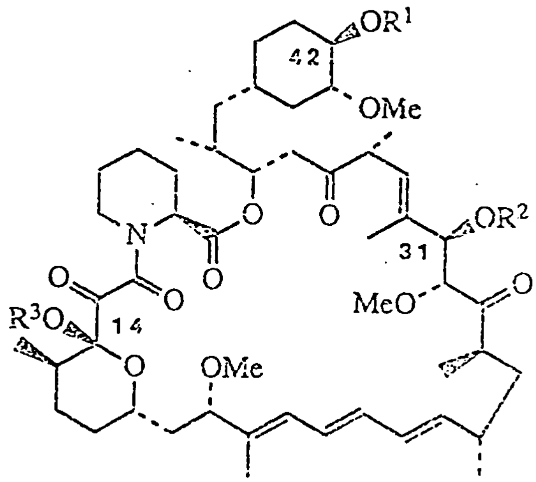
^{1}H NMR (d_6-DMSO, 400 MHz) 5.231 (m, 1 H, -CHO_2C), 4.554 (m, 1 H, MeOCHCHO_2C-), 3.426 (s, 6 H, 2 CH_3O-), 3.249 (s, 3 H, CH_3O-), 2.431 (m, 8 H, $O_2CCH_2CH_2CO_2^-$), 1.700 (s, 3 H, CH_3C=C), 1.554 (s, 3 H, CH_3C=C); ^{13}C NMR (d_6-DMSO,) 211.28 (C=O), 205.23 (C=O), 199.59 (C=O), 174.86 (C=O), 173.62 (C=O), 171.72 (C=O), 171.50 (C=O), 166.56 (C=O), 166.53 (C=O); IR (KBr) 3420 (OH), 2940 (CH), 1735 (C=O), 1630, 1580, 1460, 1400, 1380, 1170, 1070, 990 cm^{-1}; MS (neg. ion FAB) 1112 (M-1. free acid), 994, 589, 475, 297, 167, 148, 117, 99 (100).

Analysis Calcd for $C_{67}H_{109}O_{25}N_3 \cdot 2\,H_2O$	C 57.80;	H 8.12;	N 3.01
Found	C 57.91;	H 8.21;	N 2.37

CLAIMS

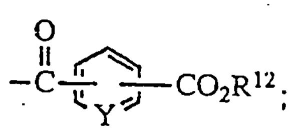
What is claimed is:

1. A compound of the structure



wherein R^1, R^2, and R^3 are each, independently, hydrogen, or R^4;

R^4 is $-[\overset{O}{\overset{\|}{C}}(CH_2)_m\underset{R^5}{\underset{|}{CH}}(CH_2)_n\underset{R^6}{\underset{|}{N}}]_pCO_2R^7$, $-\overset{O}{\overset{\|}{C}}-(CH_2)_tX(CH_2)_uCO_2R^{11}$, or

;

R^5 is hydrogen, alkyl of 1-6 carbon atoms, aralkyl of 7-10 carbon atoms, $-(CH_2)_qCO_2R^8$, $-(CH_2)_rNR^9CO_2R^{10}$, carbamylalkyl of 2-3 carbon atoms, aminoalkyl of 1-4 carbon atoms, hydroxyalkyl of 1-4 carbon atoms, guanylalkyl of 2-4 carbon atoms, mercaptoalkyl of 1-4 carbon atoms, alkylthioalkyl of 2-6 carbon atoms, indolylmethyl, hydroxyphenylmethyl, imidazoylmethyl or phenyl which is optionally mono-, di-, or tri-substituted with a substituent selected from alkyl of 1-6 carbon atoms, alkoxy of 1-6 carbon atoms, hydroxy, cyano, halo, nitro, carbalkoxy of 2-7 carbon atoms, trifluoromethyl, amino, or a carboxylic acid;

R^6 and R^9 are each, independently, hydrogen, alkyl of 1-6 carbon atoms, or aralkyl of 7-10 carbon atoms;

R^7, R^8, and R^{10} are each, independently, alkyl of 1-6 carbon atoms, aralkyl of 7-10 carbon atoms, fluorenylmethyl, or phenyl which is optionally mono-, di-, or tri-substituted with a substituent selected from alkyl of 1-6 carbon atoms, alkoxy of 1-6 carbon atoms, hydroxy, cyano, halo, nitro, carbalkoxy of 2-7 carbon atoms, trifluoromethyl, amino, or a carboxylic acid;

R^{11} and R^{12} are each, independently, alkyl of 1-6 carbon atoms, aralkyl of 7-10 carbon atoms, or phenyl which is optionally mono-, di-, or tri-substituted with a substituent selected from alkyl of 1-6 carbon atoms, alkoxy of 1-6 carbon atoms, hydroxy, cyano, halo, nitro, carbalkoxy of 2-7 carbon atoms, trifluoromethyl, amino, or a carboxylic acid;

X is $-\underset{R^{14}}{\overset{R^{13}}{\underset{|}{\overset{|}{C}}}}-$, O, or S;

R^{13} and R^{14} are each, independently, hydrogen or alkyl of 1-6 carbon atoms;

Y is CH or N;

m is 0 - 4;

n is 0 - 4;

p is 1 - 2;

q is 0 - 4;

r is 0 - 4;

t is 0 - 4;

u is 0 - 4;

wherein R^5, R^6, m, and n are independent in each of the $[\overset{O}{\overset{\|}{C}}(CH_2)_m\underset{R^5}{\underset{|}{C}}H(CH_2)_n\underset{R^6}{\underset{|}{N}}]$ subunits when p = 2;

or a pharmaceutically acceptable salt thereof, with the proviso that R^1, R^2, and R^3 are not all hydrogen, further provided that R^1, R^2, and R^3 are not all

$-[\overset{\overset{\displaystyle O}{\|}}{C}(CH_2)_m\underset{\underset{\displaystyle R^5}{|}}{CH}(CH_2)_n\underset{\underset{\displaystyle R^6}{|}}{N}]_pCO_2R^7$, and still further provided that t and u are not both 0 when X is O or S.

2. A compound of claim 1 where R^4 is $-[\overset{\overset{\displaystyle O}{\|}}{C}(CH_2)_m\underset{\underset{\displaystyle R^5}{|}}{CH}(CH_2)_n\underset{\underset{\displaystyle R^6}{|}}{N}]_pCO_2R^7$, $m = 0$, $n = 0$, and $p = 1$ or a pharmaceutically acceptable salt thereof.

3. A compound of claim 1 where R^4 is $-[\overset{\overset{\displaystyle O}{\|}}{C}(CH_2)_m\underset{\underset{\displaystyle R^5}{|}}{CH}(CH_2)_n\underset{\underset{\displaystyle R^6}{|}}{N}]_pCO_2R^7$, $m = 0$, $n = 0$, and $p = 2$ or a pharmaceutically acceptable salt thereof.

4. A compound of claim 1 where R^4 is $-[\overset{\overset{\displaystyle O}{\|}}{C}(CH_2)_m\underset{\underset{\displaystyle R^5}{|}}{CH}(CH_2)_n\underset{\underset{\displaystyle R^6}{|}}{N}]_pCO_2R^7$, $n = 0$, and R^5 is $-(CH_2)_qCO_2R^8$ or a pharmaceutically acceptable salt thereof.

5. A compound of claim 1 where R^4 is $-[\overset{\overset{\displaystyle O}{\|}}{C}(CH_2)_m\underset{\underset{\displaystyle R^5}{|}}{CH}(CH_2)_n\underset{\underset{\displaystyle R^6}{|}}{N}]_pCO_2R^7$, $m = 0$, $n = 0$, and R^5 is $-(CH_2)_rNR^9CO_2R^{10}$ or a pharmaceutically acceptable salt thereof.

6. A compound of claim 1 where R^4 is $-[\overset{\overset{\displaystyle O}{\|}}{C}(CH_2)_m\underset{\underset{\displaystyle R^5}{|}}{CH}(CH_2)_n\underset{\underset{\displaystyle R^6}{|}}{N}]_pCO_2R^7$, $m = 0$, $n = 0$, and R^5 is hydrogen or a pharmaceutically acceptable salt thereof.

7. A compound of claim 1 where R^4 is $-\overset{\overset{\displaystyle O}{\|}}{C}-(CH_2)_tX(CH_2)_uCO_2R^{11}$ or a pharmaceutically acceptable salt thereof.

8. A compound of claim 1 which is rapamycin-42-ester with N-[(1,1-dimethylethoxy)carbonyl]-glycylglycine or a pharmaceutically acceptable salt thereof.

9. A compound of claim 1 which is rapamycin-31,42-diester with N-[(1,1-dimethyl- ethoxy)carbonyl]-glycylglycine or a pharmaceutically acceptable salt thereof.

10. A compound of claim 1 which is rapamycin-31,42-diester with N-[(1,1-dimethylethoxy)carbonyl]-N-methylglycine or a pharmaceutically acceptable salt thereof.

11. A compound of claim 1 which is rapamycin-42-ester with N-[(1,1-dimethylethoxy)carbonyl]-N-methylglycine or a pharmaceutically acceptable salt thereof.

12. A compound of claim 1 which is rapamycin-31,42-diester with 5-(1,1-dimethylethoxy)-2-[[(1,1-dimethylethoxy)carbonyl]amino]-5-oxopentanoic acid or a pharmaceutically acceptable salt thereof.

13. A compound of claim 1 which is rapamycin-42-ester with 5-(1,1-dimethylethoxy)-2-[[(1,1-dimethylethoxy)carbonyl]amino]-5-oxopentanoic acid or a pharmaceutically acceptable salt thereof.

14. A compound of claim 1 which is rapamycin-31,42-diester with 2-[[(1,1-dimethylethoxy)carbonyl]amino]-4-oxo-4-(phenylmethoxy) butanoic acid or a pharmaceutically acceptable salt thereof.

15. A compound of claim 1 which is rapamycin-31,42-diester with 3-[[(1,1-dimethylethoxy)carbonyl]amino]-4-oxo-4-(phenylmethoxy) butanoic acid or a pharmaceutically acceptable salt thereof.

16. A compound of claim 1 which is rapamycin-42-ester with 3-[[(1,1-dimethylethoxy)carbonyl]amino]-4-oxo-4-(phenylmethoxy) butanoic acid or a pharmaceutically acceptable salt thereof.

17. A compound of claim 1 which is rapamycin-42-ester with 5-(1,1-dimethyl-oxy)-4-[[(1,1-dimethylethoxy)carbonyl]amino]-5-oxopentanoic acid or a pharmaceutically acceptable salt thereof.

18. A compound of claim 1 which is rapamycin-31,42-diester with 5-(1,1-dimethylethoxy)-4-[[(1,1-dimethylethoxy)carbonyl]amino]-5-oxopentanoic acid or a pharmaceutically acceptable salt thereof.

19. A compound of claim 1 which is rapamycin-42-ester with N^{α}, N^{ε}-bis[(1,1-dimethylethoxy)carbonyl]-L-lysine or a pharmaceutically acceptable salt thereof.

20. A compound of claim 1 which is rapamycin-31,42-diester with N^{α}, N^{ε} bis[(1,1-dimethylethoxy)carbonyl]-L-lysine or a pharmaceutically acceptable salt thereof.

21. A compound of claim 1 which is rapamycin-14,31,42-tris(monobenzylsuccinate) or a pharmaceutically acceptable salt thereof.

22. A compound of claim 1 which is rapamycin-31,42-bis(monobenzylsuccinate) or a pharmaceutically acceptable salt thereof.

23. A compound of claim 1 which is rapamycin-42-(monobenzylsuccinate) or a pharmaceutically acceptable salt thereof.

24. A compound of claim 1 which is rapamycin-31,42-bishemiglutarate or a pharmaceutically acceptable salt thereof.

25. A compound of claim 1 which is rapamycin-31,42-hemiglutarate bissodium salt.

26. A compound of claim 1 which is rapamycin-31,42-bishemiglutarate bistromethamine salt.

27. A compound of claim 1 which is rapamycin-42-hemi-3'-oxoglutarate or a pharmaceutically acceptable salt thereof.

28. A compound of claim 1 which is rapamycin-31,42-bishemi-3'-oxoglutarate or a pharmaceutically acceptable salt thereof.

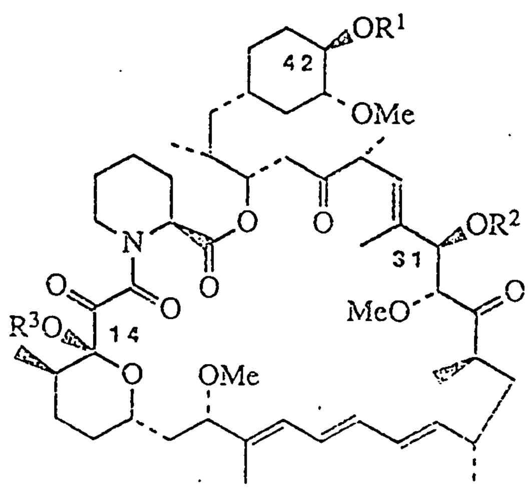
29. A compound of claim 1 which is rapamycin-31,42-bishemi-3'-oxoglutarate disodium salt.

30. A compound of claim 1 which is rapamycin-31,42-bishemi-3'-oxoglutarate bistromethamine salt.

31. A compound of claim 1 which is rapamycin-31,42-bishemisuccinate or a pharmaceutically acceptable salt thereof.

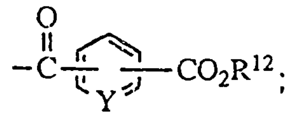
32. A compound of claim 1 which is rapamycin-31,42-bishemisuccinate bistromethane salt.

33. A method of treating transplantation rejection, host vs. graft disease, autoimmune diseases, and diseases of inflammation in a mammal by administering an immunosuppressive amount of a compound having the structure



wherein R^1, R^2, and R^3 are each, independently, hydrogen, or R^4;

R^4 is $-[\overset{O}{\overset{\|}{C}}(CH_2)_m\underset{R^5}{\underset{|}{CH}}(CH_2)_n\underset{R^6}{\underset{|}{N}}]_pCO_2R^7$, $-\overset{O}{\overset{\|}{C}}-(CH_2)_tX(CH_2)_uCO_2R^{11}$, or



R^5 is hydrogen, alkyl of 1-6 carbon atoms, aralkyl of 7-10 carbon atoms, $-(CH_2)_qCO_2R^8$, $-(CH_2)_rNR^9CO_2R^{10}$, carbamylalkyl of 2-3 carbon atoms, aminoalkyl of 1-4 carbon atoms, hydroxyalkyl of 1-4 carbon atoms, guanylalkyl of 2-4 carbon atoms, mercaptoalkyl of 1-4 carbon atoms, alkylthioalkyl of 2-6 carbon atoms, indolylmethyl, hydroxyphenylmethyl, imidazoylmethyl or phenyl which is optionally mono-, di-, or tri-substituted with a substituent selected from alkyl of 1-6 carbon atoms, alkoxy of 1-6 carbon atoms, hydroxy, cyano, halo, nitro, carbalkoxy of 2-7 carbon atoms, trifluoromethyl, amino, or a carboxylic acid;

R^6 and R^9 are each, independently, hydrogen, alkyl of 1-6 carbon atoms, or aralkyl of 7-10 carbon atoms;

R^7, R^8, and R^{10} are each, independently, alkyl of 1-6 carbon atoms, aralkyl of 7-10 carbon atoms, fluorenylmethyl, or phenyl which is optionally mono-, di-, or tri-substituted with a substituent selected from alkyl of 1-6 carbon atoms, alkoxy of 1-6 carbon atoms, hydroxy, cyano, halo, nitro, carbalkoxy of 2-7 carbon atoms, trifluoromethyl, amino, or a carboxylic acid;

R^{11} and R^{12} are each, independently, alkyl of 1-6 carbon atoms, aralkyl of 7-10 carbon atoms, or phenyl which is optionally mono-, di-, or tri-substituted with a substituent selected from alkyi of 1-6 carbon atoms, alkoxy of 1-6 carbon atoms, hydroxy, cyano, halo, nitro, carbalkoxy of 2-7 carbon atoms, trifluoromethyl, amino, or a carboxylic acid;

X is $-\underset{R^{14}}{\underset{|}{\overset{R^{13}}{\overset{|}{C}}}}-$, O, or S;

R^{13} and R^{14} are each, independently, hydrogen or alkyl of 1-6 carbon atoms;

Y is CH or N;

m is 0 - 4;

n is 0 - 4;
p is 1 - 2;
q is 0 - 4;
r is 0 - 4;
t is 0 - 4;
u is 0 - 4;

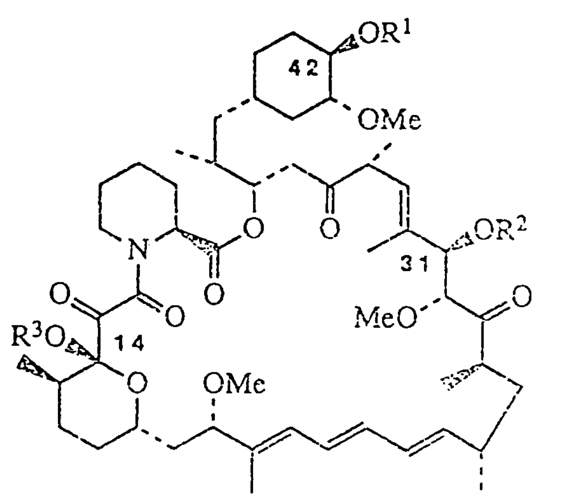
wherein R^5, R^6, m, and n are independent in each of the $[\overset{\overset{\displaystyle O}{\|}}{C}(CH_2)_m\underset{\underset{\displaystyle R^5}{|}}{CH}(CH_2)_n\underset{\underset{\displaystyle R^6}{|}}{N}]$ subunits when p = 2;

or a pharmaceutically acceptable salt thereof, with the proviso that R^1, R^2, and R^3 are not all hydrogen, further provided that R^1, R^2, and R^3 are not all

$$-[\overset{\overset{\displaystyle O}{\|}}{C}(CH_2)_m\underset{\underset{\displaystyle R^5}{|}}{CH}(CH_2)_n\underset{\underset{\displaystyle R^6}{|}}{N}]_pCO_2R^7$$

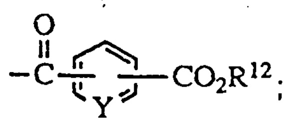
, and still further provided that t and u are not both 0 when X is O or S.

34. A method of treating fungal infections which comprises administering an antifungal amount of a compound having the structure



wherein R^1, R^2, and R^3 are each, independently, hydrogen, or R^4;

R^4 is $-[\overset{\displaystyle O}{\overset{\|}{C}}(CH_2)_m\underset{\displaystyle R^5}{\underset{|}{C}}H(CH_2)_n\underset{\displaystyle R^6}{\underset{|}{N}}]_pCO_2R^7$, $-\overset{\displaystyle O}{\overset{\|}{C}}-(CH_2)_tX(CH_2)_uCO_2R^{11}$, or



R^5 is hydrogen, alkyl of 1-6 carbon atoms, aralkyl of 7-10 carbon atoms, $-(CH_2)_qCO_2R^8$, $-(CH_2)_rNR^9CO_2R^{10}$, carbamylalkyl of 2-3 carbon atoms, aminoalkyl of 1-4 carbon atoms, hydroxyalkyl of 1-4 carbon atoms, guanylalkyl of 2-4 carbon atoms, mercaptoalkyl of 1-4 carbon atoms, alkylthioalkyl of 2-6 carbon atoms, indolylmethyl, hydroxyphenylmethyl, imidazoylmethyl or phenyl which is optionally mono-, di-, or tri-substituted with a substituent selected from alkyl of 1-6 carbon atoms, alkoxy of 1-6 carbon atoms, hydroxy, cyano, halo, nitro, carbalkoxy of 2-7 carbon atoms, trifluoromethyl, amino, or a carboxylic acid;

R^6 and R^9 are each, independently, hydrogen, alkyl of 1-6 carbon atoms, or aralkyl of 7-10 carbon atoms;

R^7, R^8, and R^{10} are each, independently, alkyl of 1-6 carbon atoms, aralkyl of 7-10 carbon atoms, fluorenylmethyl, or phenyl which is optionally mono-, di-, or tri-substituted with a substituent selected from alkyl of 1-6 carbon atoms, alkoxy of 1-6 carbon atoms, hydroxy, cyano, halo, nitro, carbalkoxy of 2-7 carbon atoms, trifluoromethyl, amino, or a carboxylic acid;

R^{11} and R^{12} are each, independently, alkyl of 1-6 carbon atoms, aralkyl of 7-10 carbon atoms, or phenyl which is optionally mono-, di-, or tri-substituted with a substituent selected from alkyl of 1-6 carbon atoms, alkoxy of 1-6 carbon atoms, hydroxy, cyano, halo, nitro, carbalkoxy of 2-7 carbon atoms, trifluoromethyl, amino, or a carboxylic acid;

X is $-\overset{\displaystyle R^{13}}{\overset{|}{\underset{\displaystyle R^{14}}{\underset{|}{C}}}}-$, O, or S;

R^{13} and R^{14} are each, independently, hydrogen or alkyl of 1-6 carbon atoms;

Y is CH or N;

m is 0 - 4;

n is 0 - 4;

p is 1 - 2;

q is 0 - 4;

r is 0 - 4;

t is 0 - 4;

u is 0 - 4;

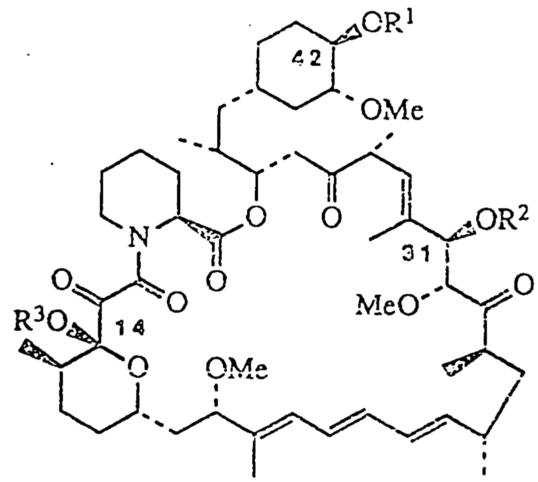
wherein R^5, R^6, m, and n are independent in each of the $\left[\overset{O}{\overset{\|}{C}}(CH_2)_m\underset{R^5}{\underset{|}{C}}H(CH_2)_n\underset{R^6}{\underset{|}{N}}\right]$ subunits when p = 2;

or a pharmaceutically acceptable salt thereof, with the proviso that R^1, R^2, and R^3 are not all hydrogen, further provided that R^1, R^2, and R^3 are not all

$$-\left[\overset{O}{\overset{\|}{C}}(CH_2)_m\underset{R^5}{\underset{|}{C}}H(CH_2)_n\underset{R^6}{\underset{|}{N}}\right]_pCO_2R^7$$

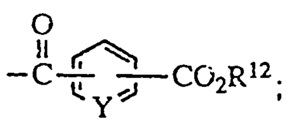
, and still further provided that t and u are not both 0 when X is O or S.

35. A pharmaceutical composition for the use in treating transplantation rejection, host vs. graft disease, autoimmune diseases, and diseases of inflammation in a mammal which comprises, an immunosuppressive amount of a compound having the structure



wherein R^1, R^2, and R^3 are each, independently, hydrogen, or R^4;

$$R^4 \text{ is } -[\overset{O}{\overset{\|}{C}}(CH_2)_m\underset{R^5}{\underset{|}{CH}}(CH_2)_n\underset{R^6}{\underset{|}{N}}]_pCO_2R^7 \;, \quad -\overset{O}{\overset{\|}{C}}-(CH_2)_tX(CH_2)_uCO_2R^{11} \;, \text{ or}$$



R^5 is hydrogen, alkyl of 1-6 carbon atoms, aralkyl of 7-10 carbon atoms, $-(CH_2)_qCO_2R^8$, $-(CH_2)_rNR^9CO_2R^{10}$, carbamylalkyl of 2-3 carbon atoms, aminoalkyl of 1-4 carbon atoms, hydroxyalkyl of 1-4 carbon atoms, guanylalkyl of 2-4 carbon atoms, mercaptoalkyl of 1-4 carbon atoms, alkylthioalkyl of 2-6 carbon atoms, indolylmethyl, hydroxyphenylmethyl, imidazoylmethyl or phenyl which is optionally mono-, di-, or tri-substituted with a substituent selected from alkyl of 1-6 carbon atoms, alkoxy of 1-6 carbon atoms, hydroxy, cyano, halo, nitro, carbalkoxy of 2-7 carbon atoms, trifluoromethyl, amino, or a carboxylic acid;

R^6 and R^9 are each, independently, hydrogen, alkyl of 1-6 carbon atoms, or aralkyl of 7-10 carbon atoms;

R^7, R^8, and R^{10} are each, independently, alkyl of 1-6 carbon atoms, aralkyl of 7-10 carbon atoms, fluorenylmethyl, or phenyl which is optionally mono-, di-, or tri-substituted with a substituent selected from alkyl of 1-6 carbon atoms, alkoxy

of 1-6 carbon atoms, hydroxy, cyano, halo, nitro, carbalkoxy of 2-7 carbon atoms, trifluoromethyl, amino, or a carboxylic acid;

R^{11} and R^{12} are each, independently, alkyl of 1-6 carbon atoms, aralkyl of 7-10 carbon atoms, or phenyl which is optionally mono-, di-, or tri-substituted with a substituent selected from alkyl of 1-6 carbon atoms, alkoxy of 1-6 carbon atoms, hydroxy, cyano, halo, nitro, carbalkoxy of 2-7 carbon atoms, trifluoromethyl, amino, or a carboxylic acid;

X is $-\overset{\displaystyle R^{13}}{\underset{\displaystyle R^{14}}{\overset{|}{\underset{|}{C}}}}-$, O, or S;

R^{13} and R^{14} are each, independently, hydrogen or alkyl of 1-6 carbon atoms;

Y is CH or N;

m is 0 - 4;

n is 0 - 4;

p is 1 - 2;

q is 0 - 4;

r is 0 - 4;

t is 0 - 4;

u is 0 - 4;

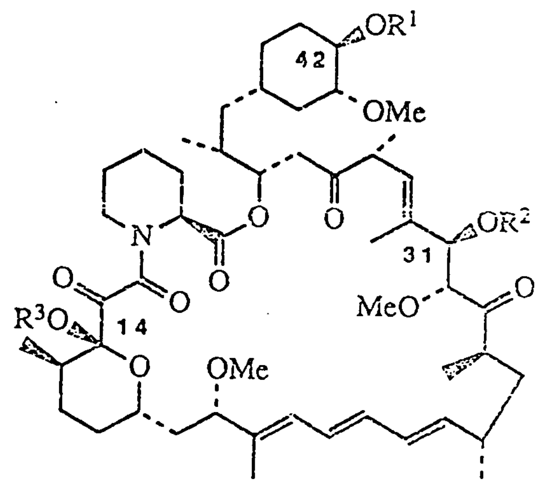
wherein R^5, R^6, m, and n are independent in each of the $[\overset{\displaystyle O}{\overset{||}{C}}(CH_2)_m\underset{\displaystyle R^5}{\underset{|}{CH}}(CH_2)_n\underset{\displaystyle R^6}{\underset{|}{N}}]$ subunits when p = 2;

or a pharmaceutically acceptable salt thereof, with the proviso that R^1, R^2, and R^3 are not all hydrogen, further provided that R^1, R^2, and R^3 are not all

$$-[\overset{\displaystyle O}{\overset{||}{C}}(CH_2)_m\underset{\displaystyle R^5}{\underset{|}{CH}}(CH_2)_n\underset{\displaystyle R^6}{\underset{|}{N}}]_pCO_2R^7$$

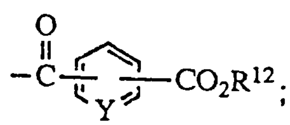
, and still further provided that t and u are not both 0 when X is O or S.

36. A pharmaceutical composition for the use in treating fungal infections, which comprises an antifungal amount of a compound having the structure



wherein R^1, R^2, and R^3 are each, independently, hydrogen, or R^4;

R^4 is $-[\overset{O}{\overset{\|}{C}}(CH_2)_m\underset{R^5}{\underset{|}{CH}}(CH_2)_n\underset{R^6}{\underset{|}{N}}]_pCO_2R^7$, $-\overset{O}{\overset{\|}{C}}-(CH_2)_tX(CH_2)_uCO_2R^{11}$, or



R^5 is hydrogen, alkyl of 1-6 carbon atoms, aralkyl of 7-10 carbon atoms, $-(CH_2)_qCO_2R^8$, $-(CH_2)_rNR^9CO_2R^{10}$, carbamylalkyl of 2-3 carbon atoms, aminoalkyl of 1-4 carbon atoms, hydroxyalkyl of 1-4 carbon atoms, guanylalkyl of 2-4 carbon atoms, mercaptoalkyl of 1-4 carbon atoms, alkylthioalkyl of 2-6 carbon atoms, indolylmethyl, hydroxyphenylmethyl, imidazoylmethyl or phenyl which is optionally mono-, di-, or tri-substituted with a substituent selected from alkyl of 1-6 carbon atoms, alkoxy of 1-6 carbon atoms, hydroxy, cyano, halo, nitro, carbalkoxy of 2-7 carbon atoms, trifluoromethyl, amino, or a carboxylic acid;

R^6 and R^9 are each, independently, hydrogen, alkyl of 1-6 carbon atoms, or aralkyl of 7-10 carbon atoms;

R^7, R^8, and R^{10} are each, independently, alkyl of 1-6 carbon atoms, aralkyl of 7-10 carbon atoms, fluorenylmethyl, or phenyl which is optionally mono-, di-, or tri-substituted with a substituent selected from alkyl of 1-6 carbon atoms, alkoxy of 1-6 carbon atoms, hydroxy, cyano, halo, nitro, carbalkoxy of 2-7 carbon atoms, trifluoromethyl, amino, or a carboxylic acid;

R^{11} and R^{12} are each, independently, alkyl of 1-6 carbon atoms, aralkyl of 7-10 carbon atoms, or phenyl which is optionally mono-, di-, or tri-substituted with a substituent selected from alkyl of 1-6 carbon atoms, alkoxy of 1-6 carbon atoms, hydroxy, cyano, halo, nitro, carbalkoxy of 2-7 carbon atoms, trifluoromethyl, amino, or a carboxylic acid;

X is $-\underset{R^{14}}{\overset{R^{13}}{\underset{|}{\overset{|}{C}}}}-$, O, or S;

R^{13} and R^{14} are each, independently, hydrogen or alkyl of 1-6 carbon atoms;

Y is CH or N;

m is 0 - 4;

n is 0 - 4;

p is 1 - 2;

q is 0 - 4;

r is 0 - 4;

t is 0 - 4;

u is 0 - 4;

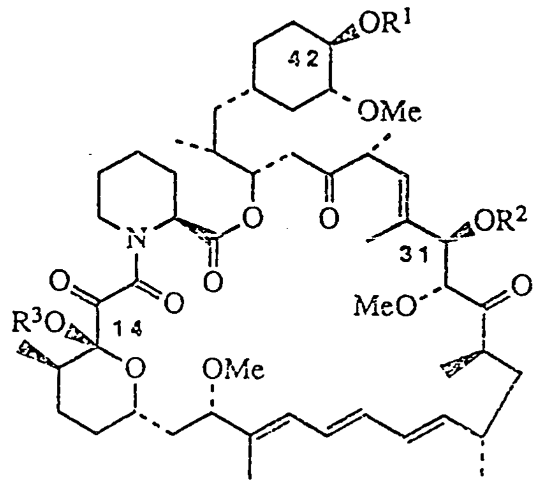
wherein R^5, R^6, m, and n are independent in each of the $[\overset{O}{\overset{\|}{C}}(CH_2)_m\underset{R^5}{\underset{|}{CH}}(CH_2)_n\underset{R^6}{\underset{|}{N}}]$ subunits when p = 2;

or a pharmaceutically acceptable salt thereof, with the proviso that R^1, R^2, and R^3 are not all hydrogen, further provided that R^1, R^2, and R^3 are not all

$$-[\overset{O}{\overset{\|}{C}}(CH_2)_m\underset{R^5}{\underset{|}{CH}}(CH_2)_n\underset{R^6}{\underset{|}{N}}]_pCO_2R^7$$

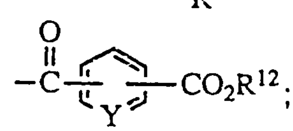
, and still further provided that t and u are not both 0 when X is O or S.

37. A process for preparing a compound of the formula



wherein R^1, R^2, and R^3 are each, independently, hydrogen, or R^4;

R^4 is $-[\overset{\overset{\large O}{\|}}{C}(CH_2)_m\underset{\underset{\large R^5}{|}}{CH}(CH_2)_n\underset{\underset{\large R^6}{|}}{N}]_pCO_2R^7$, $-\overset{\overset{\large O}{\|}}{C}-(CH_2)_tX(CH_2)_uCO_2R^{11}$, or



R^5 is hydrogen, alkyl of 1-6 carbon atoms, aralkyl of 7-10 carbon atoms, $-(CH_2)_qCO_2R^8$, $-(CH_2)_rNR^9CO_2R^{10}$, carbamylalkyl of 2-3 carbon atoms, aminoalkyl of 1-4 carbon atoms, hydroxyalkyl of 1-4 carbon atoms, guanylalkyl of 2-4 carbon atoms, mercaptoalkyl of 1-4 carbon atoms, alkylthioalkyl of 2-6 carbon atoms, indolylmethyl, hydroxyphenylmethyl, imidazoylmethyl or phenyl which is optionally mono-, di-, or tri-substituted with a substituent selected from alkyl of 1-6 carbon atoms, alkoxy of 1-6 carbon atoms, hydroxy, cyano, halo, nitro, carbalkoxy of 2-7 carbon atoms, trifluoromethyl, amino, or a carboxylic acid;

R^6 and R^9 are each, independently, hydrogen, alkyl of 1-6 carbon atoms, or aralkyl of 7-10 carbon atoms;

R^7, R^8, and R^{10} are each, independently, alkyl of 1-6 carbon atoms, aralkyl of 7-10 carbon atoms, fluorenylmethyl, or phenyl which is optionally mono-, di-, or tri-substituted with a substituent selected from alkyl of 1-6 carbon atoms, alkoxy

of 1-6 carbon atoms, hydroxy, cyano, halo, nitro, carbalkoxy of 2-7 carbon atoms, trifluoromethyl, amino, or a carboxylic acid;

R^{11} and R^{12} are each, independently, alkyl of 1-6 carbon atoms, aralkyl of 7-10 carbon atoms, or phenyl which is optionally mono-, di-, or tri-substituted with a substituent selected from alkyl of 1-6 carbon atoms, alkoxy of 1-6 carbon atoms, hydroxy, cyano, halo, nitro, carbalkoxy of 2-7 carbon atoms, trifluoromethyl, amino, or a carboxylic acid;

X is $-\underset{R^{14}}{\overset{R^{13}}{\underset{|}{\overset{|}{C}}}}-$, O, or S;

R^{13} and R^{14} are each, independently, hydrogen or alkyl of 1-6 carbon atoms;

Y is CH or N;

m is 0 - 4;

n is 0 - 4;

p is 1 - 2;

q is 0 - 4;

r is 0 - 4;

t is 0 - 4;

u is 0 - 4;

wherein R^5, R^6, m, and n are independent in each of the $[\overset{O}{\overset{\|}{C}}(CH_2)_m\underset{R^5}{\underset{|}{CH}}(CH_2)_n\underset{R^6}{\underset{|}{N}}]$

subunits when p = 2;

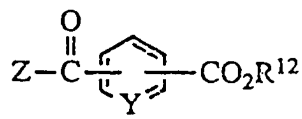
or a pharmaceutically acceptable salt thereof, with the proviso that R^1, R^2, and R^3 are not all hydrogen, further provided that R^1, R^2, and R^3 are not all

$$-[\overset{O}{\overset{\|}{C}}(CH_2)_m\underset{R^5}{\underset{|}{CH}}(CH_2)_n\underset{R^6}{\underset{|}{N}}]_pCO_2R^7$$

, and still further provided that t and u are not both 0 when X is O or S;

which comprises (a) acylating rapamycin with an acylating agent or (b) sequentially acylating rapamycin with one or more acylating agents, said acylating agent(s) being selected from acids of formula:

$$Z-[\overset{\overset{\displaystyle O}{\|}}{C}(CH_2)_m\underset{\underset{\displaystyle R^5}{|}}{C}H(CH_2)_n\underset{\underset{\displaystyle R^6}{|}}{N}]_pCO_2R^7 , \qquad Z-\overset{\overset{\displaystyle O}{\|}}{C}-(CH_2)_tX(CH_2)_uCO_2R^{11} , \text{ or}$$



where Z is OH

or reactive derivatives thereof, if desired protecting any of 42, 31 and 14 positions of rapamycin with an appropriate protecting group and removing said group as required, and further if desired isolating the product as a pharmaceutically acceptable salt.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 91/06824

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl.5 C 07 D 498/18 C 07 K 5/06 A 61 K 31/395
A 61 K 37/02 //(C 07 D 498/18 C 07 D 311:00 C 07 D 273:00

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl.5	C 07 D 498/00 C 07 H 19/00 C 07 K 5/00 A 61 K 31/00 A 61 K 37/00

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category°	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
A	EP,A,0046661 (AYERST McKENNA AND HARRISON LTD) 3 March 1982, see the whole document, & US, A, 4316885 (cited in the application) ---	1,33-36
A	US,A,4650803 (VALENTINO J.S. AND KENNEDY P.E.) 17 March 1987, see the whole document (cited in the application) -----	1,33-36

° Special categories of cited documents:[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
13-12-1991	16. 01. 92
International Searching Authority	Signature of Authorized Officer
EUROPEAN PATENT OFFICE	Daniella van der Haas

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. [X] OBSERVATION WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This International search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claim numbers because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 33-34 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compounds

2. [ ] Claim numbers because they relate to parts of the International application that do not comply with the prescribed requirements to such an extent that no meaningful International search can be carried out, specifically:

3. [ ] Claim numbers because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. [ ] OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this International application as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International search report covers all searchable claims of the International application

2. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the International application for which fees were paid, specifically claims:

3. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. [ ] As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

[ ] The additional search fees were accompanied by applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

ANNEX THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO.

US 9106824
SA 52089

...nt family members relating to the patent documents cited in the above-mentioned international search report.
...ontained in the European Patent Office EDP file on 13/01/92
...t Office is in no way liable for these particulars which are merely given for the purpose of information.

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		AT-T- E7920	15-06-84
		CA-A- 1159054	20-12-83
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		GB-A,B 2183647	10-06-87
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